

Overt Responses of *Polychoerus carmelensis* (Turbellaria: Acoela) to Abrupt Changes in Ambient Water Temperature

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KNOWLEDGE of an animal's response to a change in the ambient environment contributes to an understanding of its behavior, activity pattern, and methods of survival. Responses to environmental stimuli by triploblastic animals having a comparatively low order of tissue/organ development as in the Acoela has special evolutionary significance because, according to the hypothesis of Hadzi, they are the stem group of the Eumetazoa and were derived from ciliates (de Beer, 1954; Hanson, 1958). Of special interest are the flatworms which inhabit tide pools of the midtide horizon, where ambient temperatures may fluctuate because of shallow, relatively nonturbulent water. Such is the habitat of the Acoela flatworm *Polychoerus carmelensis* in the vicinity of Monterey, California (Ricketts and Calvin, 1952). During low tide this species is often abundantly present on algae-covered rocks. At high tide *Polychoerus* takes shelter under rocks and gravel, apparently in response to water turbulence caused by the incoming tide. Because of potentially pronounced environmental changes within its ecosystem, *Polychoerus* was selected for study of the overt responses by an exothermic marine animal to changes in the ambient water temperature.

Dr. Donald P. Abbott, Assistant Director of the Hopkins Marine Station, contributed several much-appreciated suggestions during this study.

METHODS

Specimens of *P. carmelensis* collected at Point Pinos (vicinity of the Hopkins Marine Station of Stanford University, Pacific Grove, California) were transported to the University of California Animal Physiology Laboratory at

Davis, California. One group of animals was maintained for 24 hr and another for 48 hr in a darkened chamber at a temperature of 13°–14°C. This temperature approximates that of Monterey Bay in late spring. In the following tests an individual flatworm was removed from the chamber, placed on a horizontal plastic grid, and quickly submerged to a depth of 1 cm in a controlled-temperature sea water bath. During the experiments the water temperature was maintained within $\pm 0.5^\circ\text{C}$ of the desired temperature at the upper surface of the submerged plastic grid. The amount of illumination from fluorescent room lights at water level was constant at 60 ft-c throughout all tests. This was sufficient to induce a photokinetic response from the dark-conditioned animals.

As an animal moved across the plastic grid, its horizontal movements during a 30-second period were transcribed by the author onto a record sheet grid. Such a record was obtained for each individual tested. All animals were allowed to travel a distance of about 1 cm before the record tracings were initiated. The total distance traveled, often in a highly erratic course, was measured from the record sheet grid; the rate of locomotion was computed in mm/min.

After being dark-conditioned for 24 hr, 10 individuals were tested at each of the following temperatures: 3°, 5°, 8°, 11°, 14°, 17°, 21°, 25°, 29°, 33°, 35°, and 38°C. No further tests were made on any individual, once its locomotion rate at a specific temperature was determined. On the following day, each test was repeated using 10 animals dark-conditioned for 48 hr. There was no apparent difference in the average rate of locomotion related to duration of conditioning. Therefore, the locomotion rate data for all 20 individuals tested at each specific temperature were consolidated.

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RESPONSES TO AMBIENT WATER TEMPERATURE

Reactions to Cold Temperature

P. carmelensis placed in sea water at a temperature of 3°C immediately contracted into a U-shaped posture with normally ventral portion of the animal forming the inside surface of the U. Few animals exposed to this temperature had noticeable muscular or ciliary motion while in this posture. Thus, the well-developed automaticity normally associated with ciliary motion (Prosser and Brown, 1961) apparently did not occur in *P. carmelensis* exposed to sea water at 3°C. After several minutes' exposure, disintegration of the epidermal cells occurred, and shortly thereafter a gentle motion of the water caused by stirring with a probe resulted in disorganization of the body structure.

All *polychoerus* exposed to an ambient water temperature of 5°C contracted into the U-shaped posture and were motionless for several minutes. Thereafter most were capable of locomotion (it was necessary to test 24 individuals to obtain locomotion rates for 20 animals). These animals were motile long enough to measure locomotion rates ranging from 5.0 to 32.5 mm/min with an average of 17.4 mm/min. This was the lowest average obtained in these tests. At the same temperature, 16 individuals moved about while in the U-shaped posture. Apparently this movement was accomplished entirely by motion of cilia on the dorsal surface, since no muscular contractions were noticed as the animals glided over the surface of the plastic grid. These animals moved about for only a minute or two and thereafter tissue disintegration took place as described above. The highest rates of locomotion at 5°C were obtained from 4 animals that, after a short period in the U-shaped posture, moved in the typical flatworm posture. Within a few minutes movement ceased, whereupon they again contracted into the U-shaped posture and died.

Reactions to Changes in Ambient Water Temperature

The locomotion rate of *P. carmelensis* was clearly influenced by the temperature of ambient sea water under the conditions of these experiments. However, there were pronounced changes in the manner in which locomotion

occurred. Several of the 20 animals exposed to water at 8°C began to move while in the U-shaped posture. Locomotion was accomplished by action of the dorsal cilia, the only portion of the body in contact with the plastic grid. These animals soon reoriented to the typical flatworm posture and the rate of locomotion at 8°C was measured from this posture only. At this temperature *Polychoerus* traveled at an average rate of 44.9 mm/min. Accelerated locomotion rates associated with increases in ambient water temperatures were measured at 11°, 14°, and 17°C with average values of 64.8, 83.0, and 90.4 mm/min respectively. Thus, the average rate of locomotion for *P. carmelensis* acclimated at 13°–14°C increased from 17.4 to 90.4 mm/min in response to a 12-degree rise (from 5° to 17°C) in temperature. This increase in speed of locomotion took place at a nearly uniform rate of 6.1 mm/min/°C increase in water temperature (see Table 1).

P. carmelensis specimens respond to temperatures higher than 17°C by decreasing their rate of locomotion. A reduction in average locomotion rate was measured at 21°C (78.5 mm/min), 25°C (66.2 mm/min), 29°C (50.2 mm/min), and 33°C (32.8 mm/min). This results in a steady decrease in the speed of locomotion at an approximate rate of 4.4 mm/min/°C rise in temperature between 17° and 33°C.

Reactions to Warm Temperatures

At an ambient water temperature of 29°C *Polychoerus* usually contracted into a curled position with the posterior portion of the body drawn up under the more anterior portion. Locomotion in this posture was primarily accomplished by action of the anterior portion of the body, since much of the posterior portion was not in contact with the plastic grid. The animals had an average locomotion rate of 50.2 mm/min while in this posture and, although several animals died after 5–10 minutes' exposure, it is reasonable to assume that they would have found a more suitable temperature within this length of time in their natural habitat.

Most of the individuals exposed to an ambient water temperature of 33°C immediately contracted into the curled posture mentioned above and were capable of locomotion for only

TABLE 1
REACTIONS OF *P. carmelensis* TO CHANGES IN AMBIENT WATER TEMPERATURE*

TEMPERATURE (°C)	LOCOMOTION RATE (MM/MIN)		STANDARD DEVIATION	STANDARD ERROR OF THE MEAN
	Average	Range		
3**				
5	17.4	5.0– 32.5	6.83	1.51
8	44.9	25.0– 62.5	9.81	2.19
11	64.8	40.0– 90.0	14.21	3.18
14	83.0	62.5–115.0	14.70	3.25
17	90.4	57.5–122.5	17.06	3.82
21	78.5	45.0– 95.0	13.89	3.10
25	66.2	45.0– 87.5	11.40	2.53
29	50.2	35.0– 62.5	7.41	1.65
33	32.8	17.5– 65.0	11.49	2.57
35**				
38**				

* A total of 20 individuals was tested at each of the temperatures.

** See text.

1–3 minutes. Thereafter disintegration of the tissues took place. Some of the flatworms tested at this temperature immediately formed the U-shaped posture. After about a minute most individuals reoriented to the curled posture and moved about for a minute or two. After this short period of movement they again formed the U-shaped posture and all movement ceased. Animals removed after about 3 minutes' exposure to 33°C water temperature did not recover when placed in 14°C sea water. An exposure of 5–10 minutes at this temperature results in an apparently complete disorganization of the body structure.

Exposure to sea water at a temperature of 35°C resulted in a very brief but rapid locomotion by several of the 20 individuals tested. Generally this occurred while the animal was in the curled posture. However, most individuals remained in the U-shaped posture assumed immediately upon contact with the 35°C water and had no measurable amount of locomotion. All animals showed signs of tissue disintegration within 60 seconds after exposure.

Polychaerus exposed to sea water at 38°C appeared to die immediately. Several individuals were dipped into water at this temperature and then quickly returned to 14°C sea water but there were no recoveries.

DISCUSSION

The locomotion rate of *P. carmelensis* was

clearly related to the water temperature under the conditions of these experiments. The nearly uniform increase of locomotion rate at 6.1 mm/min/°C rise in temperature between 5° and 17°C suggests that changes in tide pool temperatures may have a pronounced effect on the activity and behavior of this species. The mechanism by which temperature induces increased locomotion activity is not known. However, it is likely that this accelerated locomotion is fundamentally similar to the increases of chemical and physical reactions normally associated with an increase in temperature. Many biological processes, including rate of development, behavioral reactions, speed of locomotion, and metabolism show increases associated with higher temperatures (Prosser and Brown, 1961).

It is significant that the highest rate of locomotion occurred at a temperature (17°C) near that measured in these tide pools during late spring. This suggests that the maximum locomotion rate of *Polychaerus* may be a function of the most suitable ambient environmental temperature with respect to possible acclimatization of the animal.

The decrease in locomotion rate measured at ambient water temperatures above 17°C (4.4 mm/min/°C rise in temperature between 17° and 33°C) is considerably less than that measured for locomotion increases (6.1 mm/min/°C rise in temperature between 5° and 17°C). This suggests a temperature-related differential

response rate as well as a differential behavior response. The temperature threshold at which the type of response, increased or decreased locomotion, and the rate at which the response takes place is approximately 17°C for *P. carmelensis* accustomed to an ambient water temperature of 13°–14°C. A possible explanation for this is that accelerated locomotion results from a direct influence by the ambient temperature on body processes, and decreased locomotion results as a secondary effect of temperature-related factors, such as the reduced availability of oxygen as the ambient water temperature increases. *Polychaerus* obtains oxygen from the aquatic environment by diffusion through epidermal tissues. The physical characteristics of this species are such that sufficient oxygen for metabolic processes should be available from the environment at the higher temperature levels tested if the entire surface of the animal is effectively exposed to the environment. However, it is possible that little oxygen diffuses through the ventral epidermal tissues because of the close proximity of the animal to the surface on which it crawls. This would reduce by nearly one-half the effective diffusion surface and is a possible explanation for the curled position resulting from elevated temperatures in that this position exposes about one-half of the ventral epidermal tissue to the oxygen-bearing environment.

Locomotion of *P. carmelensis* at temperatures from 8° to 25°C inclusive takes place in the typical flatworm-type posture. The highest individual rate of locomotion obtained during these tests (2.08) was noted from a worm tested at 17°C. The lowest average locomotion rate in this temperature range was 0.75 at 8°C, and the highest average of 1.50 occurred at 17°C. These locomotion rates are expressed in mm/sec and were measured at a light intensity of 60 ft-c. They compare closely with the locomotion rates reported by Armitage (1961) of 0.86 mm/sec and 1.34 mm/sec measured at illumination levels of 6 and 37 ft-c, respectively. However, he states that the behavior of *Polychaerus* was highly erratic during his locomotion rate tests, in that some individuals spent considerable time turning the head from side to side and others ceased crawling before reaching the end of a 5-cm course. A possible explana-

tion of this erratic activity, based on the temperature-locomotion rate relationship obtained in my experiments, is that the water in the petri dish used by Armitage in his tests became warm during the course of the observations because of warm room temperature and/or heat from the light source used to stimulate locomotion. In the present tests, none of the 120 worms tested between 8° and 25°C showed such behavioral responses. However, several of those tested at 29°C and essentially all at 33°C reacted in the erratic manner described by Armitage.

Survival Value of Temperature-Locomotion Relationship

According to Armitage (1961), *P. carmelensis* does not possess a persistent diurnal rhythm. Therefore, this species must depend on an environmental "cue" or a combination of such environmental stimuli to regulate its daily activity pattern. Observations by Armitage indicate that light intensity and water turbulence play a pronounced role in the regulation of activity and behavior of this animal. Because of the pronounced influence on the velocity of movement resulting from slight changes in the ambient water temperature, it seems reasonable that temperature and temperature-related factors may also function as stimuli regulating activity and behavior.

Armitage postulates that on bright days the absence of *Polychaerus* from the upper surface of rocks and gravel during low tide and relatively calm water is caused by an increased negative phototropic response to high light intensity. However, on April 29, 1965, a cool but very bright day with morning sea water at about 15°C, I observed *Polychaerus* active on the upper surfaces of rocks and gravel throughout the period of low tide. This observation, made at the same location but earlier in the season than that by Armitage, documents the fact that high light intensity does not always cause a negative phototropic response, and suggests that there is more than a single factor regulating this behavior. Ambient water temperatures above 17°C cause a reduction in the rate of locomotion for animals conditioned to 13°–14°C. It is likely that this reduced rate of locomotion in response to such temperatures or

temperature-related factors corresponds to a less suitable ambient environment, and that such conditions in their natural environment may cause *P. carmelensis* to vacate the upper surfaces of rocks and gravel.

In my experiments *Polychoerus* specimens were totally incapacitated soon after exposure to ambient water temperatures above 29°C. It is reasonable to assume that environmental factors would trigger a behavioral escape mechanism should such temperatures occur in their natural habitat. The survival value of such an environmental stimulus or combination of stimuli is dependent upon the sensitivity and response of the animal to this factor or factors. Armitage reports a 55% increase in rate of crawling when the light intensity was increased 640%, and that in his tests *P. carmelensis* was negatively phototropic. Although the magnitude of the light intensity change (in ft-c or in percent increase) and the sensitivity of the animals in terms of response to this factor (rate of crawling in mm/sec or percent increase) cannot be directly compared to similar calculations with respect to temperature change and associated activity response, it seems certain that *Polychoerus* is at least as sensitive to ambient water temperature as it is to light intensity.

Costello and Costello (1938) indicate that *P. carmelensis* may be positively phototropic in that individuals showed a tendency to group on the moderately lighted side of an aquarium. Therefore, there is evidence of both positive and negative phototropic response for this species. Armitage suggested that this species may have a differential response to low and high light intensities. Whether such a light sensitivity threshold exists, or whether the behavioral evidence supporting this possibility results from a water temperature–light intensity relationship, is at present unknown.

The physical properties of water are such that the heat energy associated with even relatively high light intensities may have little immediate effect on the temperature of the tide pool. Conversely, tide pool temperatures may in time become elevated on overcast days with relatively low light intensities. Thus, it is possible that the absence of this species from tide pools on bright days as observed by Armitage may be at least in part the result of an elevated ambient

water temperature associated with high insolation, rather than the result of high illumination levels as a discrete factor. Regardless of the nature of causative stimuli, the rate of locomotion between 8° and 29°C is sufficient for *Polychoerus* to seek a more desirable situation under rocks and gravel should the tide pool environment warrant such behavior.

Environment-Induced Posture Responses

The U-shaped posture resulting from low, high, and often from moderately high ambient water temperatures appears to be the same posture described by Armitage for *Polychoerus* exposed to osmotically unsuitable salinity concentrations. Apparently this species has only the U-shaped posture response to both hypo- and hypertonic sea water, since Armitage does not mention other postures such as the curled posture observed in my experiments at certain elevated water temperatures.

During my experiments the U-shaped posture often occurred as a response to a gentle mechanical stimulus from contact with a soft-bristled brush used to transfer the animals from one container to another. The fact that this posture occurs as a result of temperature change, salinity change, and mechanical stimulus suggests that this posture is a characteristic behavior response to many undesirable environmental conditions.

Further experimentation may show that the curled posture also occurs as a response to a variety of stimuli. However, it is certain that this response is clearly associated with ambient water temperatures near the upper lethal level. It is not known if the curled-posture response occurs as a direct result of temperature perception by a discrete thermoreceptive mechanism or as a characteristic response to temperature-related environmental factors. It may be that the curled posture common at 29°C functions to increase the amount of surface area in direct contact with the aquatic environment, thus facilitating gaseous exchange, while enabling the animal to take shelter. The U-shaped posture exposes even more surface area but has no obvious survival value at temperatures above 29°C, inasmuch as the locomotion rate is low and the animal soon dies. However, this conclusion is hypothetical, since possible advan-

tages of either posture to animals exposed to high water temperatures are not known.

CONCLUSIONS

1. The ambient water temperature clearly influences both the rate and the sign of *Polychoerus*' locomotion. A rate-directional response threshold was measured at 17°C for animals conditioned to 14°C in that, as temperatures increased from 5° to 17°, the speed of locomotion increased from an average of 17.4 to 90.4 mm/min, at the rate of 6.1 mm/min/°C. Further temperature increases from 17° to 33°C caused a steady decrease in locomotion speed from an average of 90.4 to 32.8 mm/min, and the rate of reduction per degree of temperature increase, — 4.4 mm/min, was relatively low.

2. The highest average speed of locomotion (90.4 mm/min) and the greatest individual rate (122.5 mm/min) were measured at 17°C, which is near the temperature of sea water at the location and season at which these experiments were conducted. This suggests the possibility that the maximum locomotion rate is a function of the ambient water temperature with respect to possible seasonal acclimatization by the animal.

3. It is postulated that the increased rate of locomotion as temperatures changed from 5° to 17°C corresponds to a general temperature-related acceleration of the body processes. It is not known why temperature increases above 17°C cause a reduction of locomotion speed. Possibly this is a function of decreased amounts of available oxygen due to elevated water temperatures and/or crossing a critical temperature threshold for enzymatic action.

4. *Polychoerus* has a differential movement posture with respect to high and low water temperatures. At 5°C this species contracts into a U-shaped position, and movement at an average rate of 17.4 mm/min results from motion of the cilia on the dorsal surface of the animal. In ambient sea water temperatures of 29°C and

above, movement generally occurs while the animal is in the usual flatworm position, but with the posterior portion of the body drawn up under the more anterior portion. In this position the average rate of movement was 50.2 mm/min at 29°C and 32.8 mm/min at 33°C.

5. Sea water temperatures slightly below 5°C and above 29°C are not suitable for the survival of *P. carmelensis* conditioned at a temperature of about 14°C. However, the rate of locomotion at these temperatures appears sufficient to allow this species to avoid such conditions should they occur in the tide pool. Measurements of temperatures prevailing beneath the rocks and gravel in these tide pools is needed.

6. It is probable that the temperature of the ambient sea water, as well as the intensity of illumination and the turbulence of water, function as an environmental stimulus regulating the activity and behavior of *Polychoerus*.

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