

## Schooling behavior of Antarctic krill (*Euphausia superba*) in laboratory aquaria: reactions to chemical and visual stimuli

S. W. Strand and W. M. Hamner

Department of Biology, University of California, Los Angeles, California 90024-1606, USA

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**Abstract.** Antarctic krill, *Euphausia superba*, often exhibit abnormal behavior in laboratory aquaria, usually hovering in a stationary position, unresponsive to most external stimuli. In the austral summer of 1985–1986 at Palmer Station on Anvers Island, Antarctica, we provided laboratory conditions which induced *E. superba* to school in large aquaria. Captive krill swam horizontally and exhibited the full spectrum of behaviors normally displayed while schooling at sea. Schooling krill avoided visually contrasting stimuli, with avoidance distances correlated with stimulus size. Schools responded in qualitatively different ways to presentations of food, chemical compounds, and abrupt increases in light intensity. We describe the conditions necessary for aquarium schooling and discuss the importance of an appropriate social environment for displays of escape, avoidance, and feeding behaviors and of positional preference within the school.

(personal observations). Schooling appears to be a fundamental behavioral quality of krill.

It is generally accepted that schooling is a behavioral mechanism which lessens the probability of predation upon a particular member of a population relative to the probability of capture experienced by a lone individual (Radakov 1973, Neill and Cullen 1974, Major 1978, Hamner 1984, Landeau and Terborgh 1986). There are also, of course, other adaptive advantages and disadvantages associated with schooling.

The organization of krill schools, the sensory modalities used in schooling and the behavior of both schools and individuals within schools are poorly known. In fishes, the most well-studied schooling animals, vision, as well as the lateral line system, is used in the maintenance of schools. Olfaction may also be important for both schooling and fright reactions (Pitcher 1979). Krill have large eyes, and olfactory sensory receptors are important during feeding (Hamner et al. 1983). Vision, mechanoreception and olfaction may be of importance in the organization and behavior of krill schools.

Antarctic krill have been maintained in aquaria for extensive periods of time, McWhinnie and Denys 1978, 1980, Ikeda 1985, Stretch et al. 1988). Drs. Ross and Quetin have intermittently maintained krill at Palmer Station since 1981 (personal communication). Aquarium studies on feeding behavior of krill have been carried out by a number of investigators (Pavlov 1970, Antezana et al. 1982, Hamner et al. 1983, Quetin and Ross 1985, Hamner 1988). However, despite numerous attempts to create conditions conducive to schooling, no one had successfully induced krill to school in the laboratory until our 1985–1986 season at Palmer Station. The inability to create conditions suitable for schooling has severely limited the amount and quality of information which can be obtained from captive individuals. In aquarium situations with inappropriate visual cues, the behavior of krill is very different from that observed in the ocean. Indeed, an earlier study on swimming behavior of krill (Kils 1983), suggested that krill swim obliquely in the water column at an angle of about 55°.

### Introduction

The Antarctic kill *Euphausia superba* forms the major trophic link in the Southern Ocean between the seasonally high primary productivity and whales, seals, penguins and flying birds. Adult *E. superba* occur near the ocean surface in schools, aggregations of individuals oriented uniformly and swimming in a single direction (Marr 1962, Mauchline 1980, Hamner et al. 1983, Hamner 1984). These schools are sometimes erroneously referred to as “swarms”, a term for aggregations with no spatial organization, but the formation of true schools by *E. superba* was first reported by Hardy and Gunther in 1935 (cited in Marr 1962) and later by underwater observers, both Russian (Ragulin 1969) and American (Hamner et al. 1983, Hamner 1984), as well as from shipboard (Mauchline 1980, Nemoto 1983). During our underwater observations of *E. superba*, we have almost invariably encountered them in schools. Solitary individuals that we captured were almost always damaged or parasitized

The present paper describes the environmental conditions which enabled us to induce schooling by krill in aquaria. It also reports the results of a series of experiments on the responses of schooling individuals to a variety of visual and chemical stimuli which might be similar to those provided by predators. The importance of schooling behavior is discussed in the light of these experiments.

## Materials and methods

### Collection and maintenance of specimens

During the austral summer of 1985–1986, *Euphausia superba* in coastal waters off the western side of the Antarctic Peninsula were captured by towing an Isaacs-Kidd midwater trawl through schools located by fathometer. The net was towed through a school at approximately 1.5 knots for 2 min or less, in order to capture a relatively small number of krill and therefore minimize damage to captured individuals. After capture, krill were maintained on the ship in large holding tanks with flow-through seawater, which kept the water within one degree of the ambient seawater temperature (0° to 3°C).

Upon returning to Palmer Station on Anvers Island, Antarctica, the krill were placed in circular tanks, 1.75 m in diameter, filled with water to a depth of 1 m. These tanks were white inside, with the exception of a dark grey central standpipe 6.5 cm in diameter. Each tank had flow-through circulation, with water exiting through the standpipe. Water was introduced into the tanks through valved flexible hoses. This allowed us to regulate both the velocity and direction of the water entering the tanks. Schooling experiments were performed with either no water flow, or a very low (<2 liters  $\text{min}^{-1}$ ) flow to maintain water temperature.

Krill were counted by photographing the tank from directly above while they were dispersed, and then counting individuals in the picture projected onto a screen. Tanks used for schooling experiments contained about 500 individuals (489 to 520), ranging in total length from 3.1 to 5.9 cm ( $\bar{x}$ =4.84, SD=1.1).

### The visual environment

The tank with schooling krill was surrounded with white sheeting and illuminated from below the level of the tank by two 250 W tungsten photoflood lamps (3400 K) which were directed at the white ceiling, providing diffuse lighting for the tank and surrounding visual environs. Light levels were regulated by a rheostat. Investigators wore white laboratory coats and white hats. All dark objects outside the tank and within potential view of the krill were covered with white cloth. Light levels were measured with a Weston photometer, with the sensor mounted on a Lucite panel just above the surface of the water.

### Schooling experiments

Swimming speeds were measured by marking off a 1 m segment of the circular route followed by the krill (approximately 15 cm from the edge of the tank) and using a stop-watch to time their transit. Swimming speeds were measured only when 80% or more of the individuals were estimated to be swimming cohesively. The time necessary to reform a school after disruption was arbitrarily taken as the point when we estimated that 66% of the krill were swimming synchronously. Individuals were blinded by removing them from the tank, coating their eyes with opaque acrylic paint and reintroducing them into the tank within 30 s of removal. Targets for visual avoidance experiments were made from clear Plexiglas and some

were subsequently painted either white or black with quick-drying spray paint. Visual targets were suspended from a glass rod and placed in the tank in the path of the schooling krill. Avoidance distances from the targets were measured by placing a plastic ruler above the tank and sighting visually down from the edge of the target to the edge of the school. In addition, a black cylindrical model predator, 10 cm in diameter and 20 cm long was used to test visual responses. Individuals avoided the model in the same way they did a living predator, a 25 cm standard length *Notothenia gibberifrons* (Perciformes: Osteichthyes).

In order to test directional inertia of the school, we caused the school to stall by holding the predator model motionless in the tank for 2 min and then moving the model gently around the inside of the tank for one full turn. The model was then removed from sight and, after the school reformed, its direction was recorded. The direction of rotation of the predator model (clockwise or counterclockwise) was determined by the flip of a coin.

A culture of local diatoms *Nitzschia* sp. was concentrated and added to the tank in order to test the response of krill to patches of food. Concentrated *Nitzschia* sp. (100 ml) was siphoned slowly into the tank for each experiment. Filtered seawater (100 ml) was siphoned as a control. A variety of chemicals was used to test the reactions of the krill in the school to chemicals which might be associated with the activity or presence of predators. Krill extract was made by crushing two krill into 50 ml of water, which was then strained through Watman #1 filter paper. Excretory material from giant petrels was tested by dissolving 0.5 g of dried feces into 20 ml of seawater. Human urine was collected and cooled to 0°C. Ammonium chloride was mixed with seawater in a series of dilutions from 1.0 M to 10<sup>-6</sup> M. All chemicals were added to the tank as a single drop. A single drop of filtered seawater was used as a control. After each chemical was added, reactions of the krill were noted, both immediately and after 15 min. Each chemical stimulus was repeated six times. Before each introduction of a chemical, the tank was flushed for 1 h. The water flow into the tank was then turned off for 1 h before starting the next trial. Krill were induced to school with the predator model if they were not already schooling.

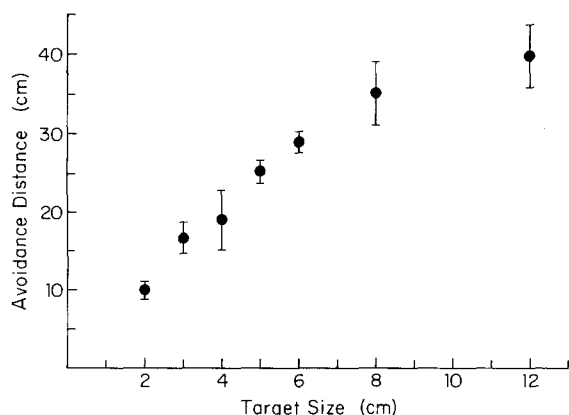
## Results

### Schooling and vision

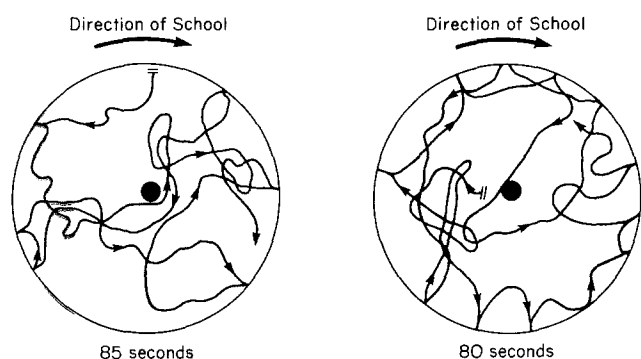
*Euphausia superba* schooled in aquaria at ambient light levels from 0.06 to 28  $\mu\text{E}$ . Schools formed most quickly at approximately 0.4  $\mu\text{E}$ . Slight or gradual variations in light level did not disturb schooling, but schooling was interrupted by sudden changes in light intensity from as little as 0.6 to 1.2  $\mu\text{E}$ . Schooling resumed spontaneously after a period of acclimation to the new light level unless the light was very bright (>28  $\mu\text{E}$ ). Schooling resumed in about 3 min after an abrupt change from 0.6 to 1.2  $\mu\text{E}$ , but could not be induced for over 2 h when the light intensity was abruptly changed from 1 to 24  $\mu\text{E}$ .

In addition to requiring appropriate levels and stability of light for schooling to occur, dark objects either within or outside the tank which were perceived by the krill caused them to move away from the object to the far side of the tank, where they ceased schooling and swarmed. Upon removal of the object from the tank or masking outside dark objects with white cloth, schooling slowly resumed, usually within 15 min. Unequal illumination of the surroundings resulted in the krill aggregating in the darker portion of the tank.

Krill schooled most cohesively in the presence of a dark standpipe in the center of the tank. The school



**Fig. 1.** *Euphasia superba*. Means and standard errors for 200 trials testing avoidance distance of schooling krill from contrasting (black) visual targets of different sizes. Filled data points = mean distances of each response. Target size = diameter of circular target or width of rectangular target



**Fig. 2.** *Euphasia superba*. Movement patterns of blind individuals in tank. During both sets of observations most of the other krill in the tank were in a cohesive, oriented school. Black circles represent central standpipe of the tank

avoided the standpipe, and remained close to the white edge of the tank, continuing to circle as long as environmental factors were not altered. Covering the dark standpipe with a white sleeve led to a slow dissolution of the school, as individuals no longer avoided the center of the tank, crossed over, and disrupted the movement of individuals on the far side.

Fig. 1 shows the distance at which the school responded to black targets in its path. There was no response to either of the control targets (clear or white matching the background). Indeed, krill collided with the control targets if they were in the way. Target shape was unimportant. Circles and squares with equal diameters/sides were avoided at equal distances. For rectangular targets, the avoidance distance was affected only by the width of the target rather than surface area (length varied from 2 to 10 times width).

Blind individuals (eyes destroyed during net capture, or eyes coated with black paint) were unable to maintain their position within the school. Their coloration became reddish, they fed continuously, and swam more slowly than did schooling individuals. Their movements within the tanks were erratic and not affected by the school

except when they collided with schooling individuals. They did not respond to any of the targets or the predator model (Fig. 2). Individuals did not react to targets or the predator model for more than 2 h after the light intensity was raised abruptly from 1 to 24  $\mu$ E. When the whole school was startled by a sudden increase in light intensity (within a few seconds), all individuals turned red, dropped to the bottom of the tank, and swam erratically until they acclimated to the new light intensity.

### Schooling and water movement

Krill schooled most readily when there was no water flow in the tank. Directional water flow disrupted schooling behavior. Turbulence generated by pouring water into the tank or by stirring with a transparent rod to which the krill did not react visually also resulted in localized and temporary disruption of schooling.

### Direction of schooling

There was no preferred direction of movement (clockwise or counterclockwise) for the school. The school could be halted by the introduction of the predator model, and then chased in either direction with the model. In 10 out of 10 trials, the krill continued to school for at least 15 min in the same direction in which they had been chased for one revolution by the predator model.

### Reactions to chemical stimuli

The addition of 100 ml of concentrated diatoms to the tank (siphoned slowly to avoid turbulence) caused individuals that encountered the food to slow down and begin feeding. However, following individuals bumped into the feeding krill and caused them to move out of the patch of food, and they then rejoined the school. The addition of several liters of concentrated diatoms to the tank caused a reduction of swimming speed and increased feeding activity, and the krill often stopped schooling.

The addition of krill extract to the tank caused the individuals that encountered it to tail-flip or swim away at higher velocity in a new direction. The krill then stopped schooling and moved to the bottom of the tank where they milled around rapidly. Approximately 4 min after the krill extract had been added, the krill moved back toward the surface and spontaneously resumed schooling, initially swimming about 50% faster than before introduction of the extract (Fig. 3). Adding krill extract to a tank of light-stunned krill caused a dramatic increase in activity level and tail-flipping, with individuals darting in all directions, but light-stunned krill did not reform a school.

The addition of urine, uric acid, and ammonium chloride caused the school to disperse, with the individuals sinking or swimming slowly to the bottom of the tank where they slowly moved about asynchronously. Schools

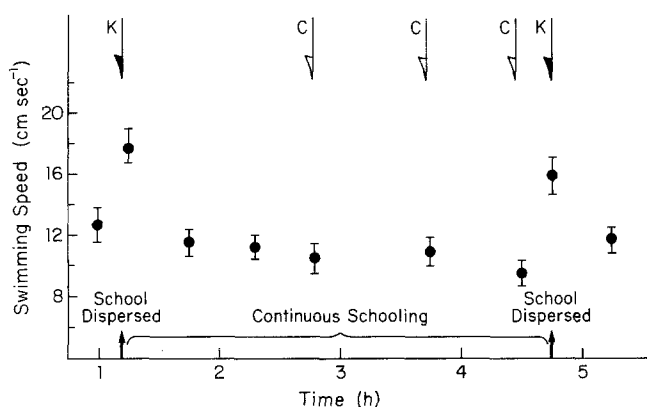


Fig. 3. *Euphausia superba*. Changes in schooling speed initiated by introduction of krill extract (K) to experimental individuals. C: addition of seawater control. Each data point (filled circle) and vertical bar represents mean speed and standard error for 50 individuals

that were dispersed by these chemicals did not reform spontaneously, and the krill remained at the bottom until the tank was flushed and the krill agitated by the predator model. Krill schools were tested with concentrations of ammonium chloride from  $1.0 M$  to  $10^{-6} M$ . Krill did not respond at  $10^{-6} M$ . One drop of  $10^{-5} M$  ammonium chloride was perceived by the krill, because all individuals in the school briefly slowed down on encountering the stimulus, but the krill quickly resumed swimming and after several minutes the odor was apparently too dispersed to elicit a response. One drop of  $10^{-4} M$  or more concentrated ammonium chloride caused the entire school to disperse and sink to the bottom of the tank.

## Discussion

The photic environment of free-living *Euphausia superba* is relatively uniform and ambient light changes relatively slowly. Changes in water movement and turbulence are common only around large objects such as icebergs or organisms larger than krill (the majority of which eat krill), and the ambient flow regimen that krill experience is, like the light regimen, relatively constant. We suggest that the creation of uniform light and water conditions in the laboratory which might approximate those of the ocean enabled us to induce krill to school in captivity. Certainly, uneven optical or flow conditions inhibit schooling in the laboratory. Schooling only occurred regularly when the visual environs outside the tank matched the walls of the tank. Any break in the white sheeting surrounding the tank or the addition of contrasting shapes in the visual field of the krill caused a disruption of the school, with the krill moving away from the contrasting object and forming a dense and non-oriented aggregation on the far side of the tank. The dark standpipe in the center of the tank aided in maintaining schooling behavior, as krill avoided it and swam in a continuous band around the tank.

In the ocean, large contrasting objects cause evasive movements by krill schools. Schools will not approach a

SCUBA diver closer than about 1 to 2 m, nor will schools pass directly below the shadow of small boats or directly above SCUBA divers. Laboratory tanks large enough for these kinds of avoidance distances were not available, but schools did not reform when large contrasting objects were present either inside or outside the tank.

The reluctance of krill to school in turbulent water might be due to confusing rheotactic information from artificial turbulence, as opposed to the normal turbulence generated within the school, which is perhaps necessary to maintain position within the school. Local turbulence, created by pouring a liter of water quickly into the tank or by stirring the tank with a transparent paddle, resulted in highly localized school disruption and quick reformation of the school. Strong directional water flow from the inlet hose in the tank inhibited the formation and maintenance of schooling.

In the laboratory, uniform lighting was accomplished by having the environment outside the tank lit uniformly and diffusely. Experimental light intensities were well within the ranges of illumination encountered by krill at sea. At sea, using SCUBA, we have observed krill schooling both at the surface and at depth on moonless nights as well as in bright sunlight. Their responses to changes in laboratory illumination are probably not due to any absolute light levels but rather to the rate and degree of change in illumination. The reactions of krill to sudden changes in light intensity may be due to their being temporarily blinded, or may be purely visual and similar to the response initiated by the appearance of a large predator. Sudden large changes in light level apparently resulted in temporary blindness of the krill, a cessation of all schooling activity, and inability to see dark objects placed in the tank. However, changes in light intensity over the same range, but occurring over a several-minute interval, also disrupted schooling but did not cause a loss of vision. It appears that the crustacean eye (or at least the eyes of *Euphausia superba*) cannot accommodate to extremely abrupt changes in light intensity.

Addition of food to the tank also affected schooling behavior. When only a small amount of food was added, individuals slowed and fed as they encountered the food-rich water. However, as they swam out of the small patch of food, they resumed rapid swimming and schooling. With the addition of larger amounts of food, the entire school stalled and, as all the individuals in the school began to feed, the direction of swimming was no longer synchronous. Individuals swam more slowly while feeding, and groups of feeding krill in the laboratory tank were less cohesive than groups of feeding krill that we have observed in the sea (Hamner et al. 1983, Hamner 1984). Schools of *Euphausia superba* in the ocean usually maintain their overall cohesiveness and directionality while feeding, even though they are spaced more widely and swim more slowly.

Krill extract, which presumably simulated a change in the chemical environment similar to that caused by a feeding predator, caused schooling to break down as individuals broke formation and engaged in escape behaviors such as rapid non-synchronous swimming and tail-flipping. School reformation after the initial reaction was

relatively quick, and the reformed school swam rapidly and was very cohesive. We interpret this coordinated high-speed schooling after disruption by "Schreckstoff" to be an adaptation for collectively leaving a site where krill predation has occurred.

The reactions of krill schools in the laboratory were qualitatively different to nitrogenous compounds than to krill extract. When nitrogenous compounds were present in high enough concentrations the krill sank slowly to the bottom of the tank and swam slowly and asynchronously. Thereafter, they did not spontaneously reform a new school, but remained at the bottom of the tank until the tank, and presumably the odors, were flushed. This behavior may be an adaptation that takes individuals out of the center or the back of a dense krill school where ammonia concentrations produced by the krill have reached intolerable levels. Moving to the bottom of a school in which ammonia concentrations are high puts the stressed individual once again into contact with clean water at the edge of the school. We suspect that movements of individual krill within a school are affected not only by increasing concentrations of excretory products toward the center and back of the school, but probably also by decreasing amounts of oxygen and food. If this is so, then continuous readjustment of position of individuals within a school should occur. Certainly it must be disadvantageous to be always at the back of the school. Although it is also possible that this response to nitrogenous compounds is a predator-avoidance behavior, the fact that both ammonia (the excretory product of krill) and vertebrate excretory compounds elicit the same response, which is different from that elicited by other types of "predator stimuli", leads us to prefer our first hypothesis. Further testing of these ideas in the laboratory and *in situ* are needed.

Krill predators range in size from copepods (e.g. *Euchaeta* sp. that feed on krill larvae; J. Yen personal communication) to blue whales. Avoidance of predators must therefore include behaviors to minimize the risk of predation by very large predators, which consume major portions of schools, and by smaller predators which take individuals one at a time. The responses of krill schools in the laboratory to models of different sizes suggests that the size of a given predator affects the distance at which the krill initiate avoidance. We believe that large dark objects outside the tank stimulate the same avoidance response as do large filter-feeding predators, resulting in a breakdown of schooling. Smaller objects seen by the krill were avoided at distances which presumably relate to the relative danger of predators in that size class. Responses of krill schools to both visual and olfactory stimuli may prove to be an interesting model with which to compare the better-studied schooling of fishes.

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