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Overcoming Obstacles - Biomimetic Lessons from the Swarming Behavior of Artemia Franciscana

Abstract

We investigated the formation of *Artemia franciscana* swarms of freshly hatched instar I nauplii larvae. Nauplii were released into light gradients but then interrupted by light-direction changes, small obstacles, or long barriers. All experiments were carried out horizontally. Each experiment used independent replicates. Freshly produced *Artemia* broods were harvested from independent incubators thus providing true replicate cohorts of *Artemia* subjected as replicates to the experimental treatments. We discovered that *Artemia* nauplii swarms can: 1. repeatedly react to non-obstructed light gradients that undergo repeated direction-changes and do so in a consistent way, 2. find their way to a light source within maze-like arrangements made from small transparent obstacles, 3. move as a swarm around extended transparent barriers, following a light gradient. This paper focuses on the recognition of whole-swarm behaviors, the description thereof and the recognition of differences in whole-swarm movements comparing non-obstructed swarming with swarms encountering obstacles. Investigations of the within-swarm behaviors of individual *Artemia* nauplii and their interactions with neighboring nauplii are in progress, e.g. in order to discover the underlying swarming algorithms and differences thereof comparing non-obstructed vs. obstructed pathways.

Keywords: Artemia production, swarming in light gradients, mazes and obstacles, high speed videography, adaptations to natural environments

1. Introduction

Vertical swarming in *Artemia* species (Crustacea, Anostraca) can be observed in the lab and is expected to constitute adaptive and useful behavior in their natural aquatic environment. Swarming is suggested to be primarily motivated by the feeding status of the *Artemia* and in response to gradients of salinity, oxygen, and light [1]. In the lab,

swarming of *Artemia* has been shown to vary with population density and age [2]. Positive swarming within point-source light-gradients is most intensive in freshly hatched nauplii and diminishes gradually towards adulthood. *Artemia* are known to readily respond to light with group motion, while they seem not influenced by electro-magnetic fields, DC voltage or thermal gradients [3]. Nauplii will not swarm towards a light source unless a critical group-density is reached [2]. Non-directional light that is uniformly distributed does not result in swarming [4]. *Artemia* nauplii encounter two major challenges when moving as a swarm towards a light source: they need to avoid collision with each other, which is a kind of overcoming obstacles within the swarm, and they need to orient themselves towards the light. Each individual may seek the most direct way towards the most intense light, moving parallel to neighbors avoiding collision [4]. Collisions are predicted to be more likely with decreasing distance from the light, the density of the swarm increases with decreasing distance from the light source.

An understudied question is if *Artemia* nauplii, can navigate in light gradients featuring obstacles and barriers on their path. We set up point-source light gradients with and without obstacles and barriers, testing the null hypothesis of non-random responses with replicated and independently grown experimental swarms, expecting to reject the null hypothesis of randomness.

In natural waters, radiation gradients during the day, are primarily oriented in the vertical, however, natural light in water bodies is never equivalent to a point source. Natural swarms have been described to have two alternative geometries: several vertical bands of swarming *Artemia* that are interconnected into larger structures or alternatively, horizontal strings that hover right beneath the surface. Both swarm-architectures originate near the surface, where light is most intense [5].

In nature, Artemia is expected to show tendencies of moving towards and staying close to the surface - the site of food production. *Artemia* nauplii non-selectively ingest any small particles in their path, mainly planktonic bacteria and small unicellular eukaryotes [5]. There is incentive to seek the surface and stay there. However, the necessity to reside at the surface eventually leads to crowding and competition. Crowd-avoidance behaviors are likely. Aside from finding food, Artemia needs to maintain an oxygenated environment. It is known that crowding leads to low oxygen concentrations within swarms which may limit swarm density [5], so expansion and dilution of the swarms are expected at the surface. In contrast, negative conditions, like exposure to dangerous radiation, like UV-B, may maintain dense swarms for the benefit of shading.

Artemia is the most salinity-tolerant multicellular animal, a recent study disclosed details on the genetic basis of the amazing capacity for osmoregulation from freshwater to 50% salinity in *Artemia franciscana* [6]. *Artemia* is the sole macro-planktonic inhabitant of salty lakes [7]. *Artemia* are globally distributed only across aquatic environments that reach the necessary extremely high salinities. Here *Artemia* are the main predator of plankton and are released from competition and higher order predators that do not tolerate the osmotic extremes.

In contrast to the situation in nature, Artemia raised in hatcheries as food for

juvenile fish and applied to transfer supplemental nutrition, a method called bio-encapsulation [8]. In captivity Artemia do encounter predators. In fact, *Artemia* are guided by light gradients to force horizontal swarming to the location of consumption. At the feeding site, consumption is less efficient because swarming eventually hinders efficient predation. Research into proper management is needed [9] [10]. A wide spectrum of organisms, including most wild zooplankton populations [11], but also fish and bacteria have been shown to swarm as a predation escape. While the concept of swarming as a predation escape is established overall it is not expected to be relevant in wild *Artemia*.

Six sexual species of *Artemia* are now recognized, together with a heterogeneous group of parthenogenetic populations, under the binomen *Artemia parthenogenetica* [12]. Globally, Artemia are only known from biotopes with extreme salinities, other variables (temperature, light intensity and primary food production may have an influence on the sizes of the *Artemia* population, or even cause a temporary absence. Artemia are found in hundreds of lakes and salterns scattered across the globe in tropical, subtropical, and temperate climates. The salt environments are varied in ionic composition, including chloride, sulphate or carbonate. *Artemia* are present at all altitudes, from sealevel to 4500m (Tibet).

Little consensus exists on the adaptive value of swarming in Artemia nauplii in their natural habitat. Some extreme swarming is related to viral and bacterial infections when *Artemia* are manipulated to form dense swarms to enhance the transfer to the final hosts, primarily water birds that feed on zooplankton, e.g. Flamingos. In seafood farms, many such transfers have been reported. Farmed fish often get infected by feeding on wild-harvested and/or cultured *Artemia* that carry parasites, a serious economic hardship awaiting preventive solutions.

One possible benefit of vertical swarming in natural *Artemia* populations may be that the internal swarm-motion transports food or continuously replenishes oxygen in *Artemia* groups. Vertical *Artemia* swarms have been described to cause a kind of convection cell that maintains continuous water flow, like a conveyer belt, through the swarm (CK own observations).

Little is known about horizontal swarming. A potential trigger for natural horizontal swarming could be an uneven distribution of planktonic food and the need to move horizontally between patches. Such movements are easier done in a group than as individuals. Individuals within moving swarms generally benefit from the reduced energetic costs often also accompanied by improved oxygen consumption rates [13], [14], [15].

This paper investigates the swarm-behavior of Artemia instar-nauplii under laboratory conditions within directional, point-source light-gradients with and without obstracles. Overall we predicted non-random responses at the whole-swarm level. We chose light to generate swarms based on previous observations and recommendation, *Artemia* nauplii are unlikely to swarm under non-directional light conditions or in the dark [1].

In all experiments we filmed *A. franciscana* nauplii with high speed cameras for the opportunity to analyze on two spatial levels, the entire swarm and the movements of individual *Artemia* nauplii. Nauplii are anatomical different from adult Artemias.

We predict that nauplii show strong affinity to light and are able to move around obstacles in their path. Obstacles are assumed to be common in nature and strategies to navigate them should have evolved in *Artemia*. The here presented results report exclusively on the experiments featuring horizontal swarms of instar I nauplii.

2. Material and methods

2.1. *Artemia* production

The setup to produce *Artemia* nauplii consisted of 12 two-liter plastic bottles (Soda bottles, SPRITE). From six bottles the lower third had been cut off and from the remaining six the upper third was removed. This resulted in the assemblage of six units each made of two partial bottles: one bottomless head-down placed within one head-less. The growing medium per each unit consisted of 1 liter of distilled water with 25 g table salt (not iodized) added. In addition, 7 grams of baking soda were added to approximate normal seawater hardness and pH. Each unit was supplied with a plastic pipe (25 mm inner diameter) attached to tubing to allow air to be pumped into the medium by one 200 L serving 3 setups. Hereby it is important to place the pipe into the bottle-cap of the inner bottle in order to have stable aeration. The *Artemia* cyst-eggs were purchased online and wre imported from saltern facilities in the San Francisko bay arae, thus the species was *Artemia franciscana*. All cysts came from the same container to assure identical origin of the swarming nauplii used in all experiments. Each cultivation bottle received 1 g of egg-cysts. The vigorous bubble-generation at the bottom of the head-down bottle provided water circulation for constant distribution of the eggs throughout the entire medium. Incubation time varied between 46 and 50 hours, temperature was 23 – 25 degrees Celsius. All six units were placed into a plastic box and two Terra-Grow UV tube-lights were placed over each row of 3 incubator units, at a distance of 15 cm from the top of the open end of the inner incubator bottle.

2.2. *Artemia* development testing

Before the harvest, each incubator was sampled to evaluate the developmental state of the *Artemia* nauplii by taking a sample directly from the well mixed incubator bottles. Upon that four broods were selected based on high similarity and high proportions of Instar I nauplii compared to other developmental stages (unhatched eggs, umbrella-stage hatching nauplii, or nauplii that already miss their yolk sack) Instar I nauplii do not take up food as their digestive system is not functional yet. They are supported completely by yolk reserves. We always had four valid and sufficiently similar broods for all experiments reported here.

2.3. *Artemia* harvest, storage, and preparation for experiments

The harvest of the nauplii was as follows: the selected broods where left for 10 minutes without circulation so that the negatively buoyant nauplii sink to the bottom while unhatched eggs and egg shells float to the surface. Nauplii accumulate in the bottle neck of the brood container and were carefully drained by unscrewing the cap, and releasing the nauplii into plastic dishes, kept in a place without non-directional or artificial light. Immediately before use in any experiments, a strong white-light source illuminated one corner of the currently used brood-specific container which resulted in a quick gathering of active, healthy and swarming nauplii within that corner. Provided that we harvested about the same amount of nauplii from each bottle, we assumed that the same number of drops of nauplii culture from a standardized glass-pipette would assure similar densities of nauplii used in each experimental trial.

2.4. Experimental conditions and sample hierarchy

Incubation, holding-tank and experimental-tank conditions were monitored for consistent temperature and water chemistry, including nitrite, nitrate, pH, salinity, and water hardness. After *Artemia* nauplii had been used in an experiment they were immediately and humanely killed by exposing them to water of extremely high salinity which in the wild is also their most likely death cause aside from starving. No individual nauplii was ever used in two experiments. For each of the experiments the hierarchical replication structure was as follows: four independent broods (incubation containers) were sampled once for 4 independent replicated experiments per treatment (1-3) and control (4), each featuring independently raised populations. In each experiment the three unique nauplii samples were either used in a single procedure or in a series of repeated procedures depending on the experiment-type. Multiple repeated treatments were conducted in non-obstructed channel experiments but only one treatment in experiments with the channel-maze, and barrier designs. The details for each of the three experiments are described below.

2.5. Experimental setups

2.5.1. Unobstracted channel experiments

This type of experiment is designed to reject the null hypothesis that *Artemia* nauplii move randomly within light gradients and do not show swarm formation, do not change swimming direction upon changes in light direction, and that *Artemia* exhibit random passing rates in response to repeated identical light of all treatments.

Our working hypothesis was that *Artemia* nauplii form swarms that move coordinated and non-random and that they maintain passing rates across equal treatments (see figure 1).

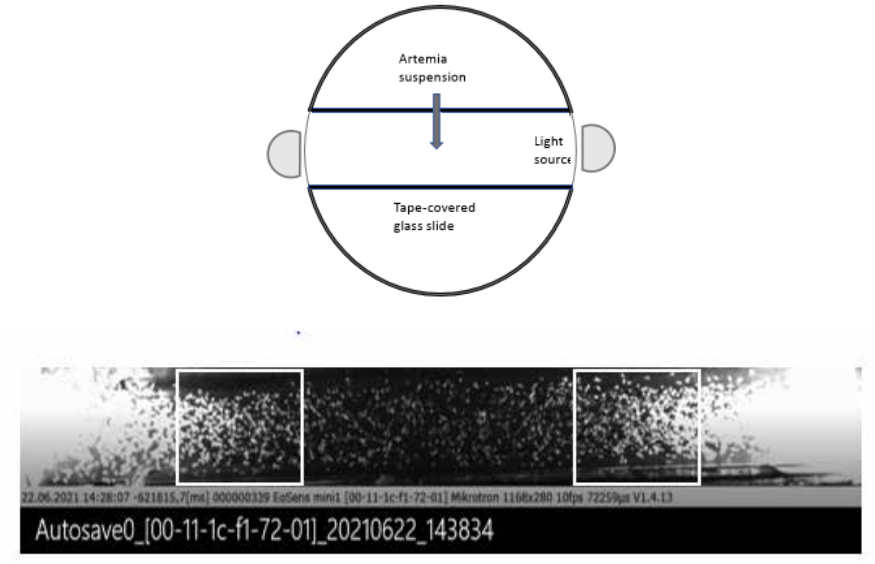

Figure 1: *A. salina* nauplii swarms in unobstracted channel experiments. *Left*: *Artemia* are added to the channel as a defined volume of concentrated *Artemia* brood to the center of the channel, using a pipette to transfer equal amounts of drops of *Artemia* nauplii brood (total of 3 unique samples per experiment) *Right*: *Artemia* organise into two swarms, one swarming to the left light source, one to the right light source. Also indicated are two counting boxes, each at an equal distance from the alternative light sources. *Artemia* entering each of the two boxes from the left and from the right are counted within standardized time periods under three different light conditions: light coming from the 1. left and right simultaneously, 2. from the left only or 3. from the right only.

Hardware

The unobstracted channel was built from a petri dish bottom, two microscope slides and some black electrical tape. The two slides are covered in black tape and positioned in parallel with a 1.5 cm gap in between. The resulting channel spans the length of the petri dish. The petri dish is entirely covered in black tape except for two clear areas at the two ends of the channel. Here the two identical strong white lights (stereo microscope lighting set) provide equal light conditions coming from each side of the channel (see Figure 1, left image). Instead of switching these lights on and off, opaque black plastic blocks have been moved in front of or removed from the light-source, this avoided inconsistencies in the spatial relation between light and channel across repeated experiments.

Procedure and statistical analysis

Ten drops of concentrated *Artemia* naupili (standardized procedure, see above) were placed in the center of the channel and were allowed to spread under equal light input applied from the left and the right ends of the channel. The expectation is that the *Artemia* added will divide into two sub-swarms moving either to the right or the left light source (see Figure 1). Rates of passing towards/away from each light source through the two boxes (see Figure 1) are estimated as the actual number of *Artemia* entering the box (from the left or from the right) within a standardized time period (total of 60 sec, counted within six separate 10 second periods, each played at 0.16 of the original video speed, using the video format MP4 in AVI (Figure 1, right image). All together, we performed three experiments using the unobstructed channel design. Each experiment was done from three independent broods. Statistical analysis has been performed on mean values, calculated from the six independent *Artemia* counts. Counts were applied in the statistical analysis without any transformation, Student t-tests were used upon assurance of equal variances (F-test).

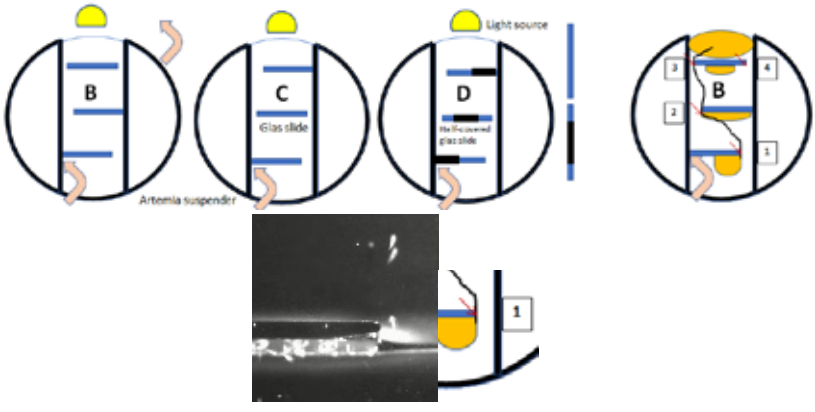

Figure 2: Three images on the left: two mazes with transparent obstacles and one with opaque obstacles. On the two far right images: the predicted movement of *Artemia* nauplii through maze B, indicates the locations at which *nauplii* are counted (red arrows) and a photo demonstrates the actual counting location, featuring nauplii in action.

2.5.2. Obstracted channel experiment – the maze

The following experiments were designed to reject the null hypothesis that *Artemia* due to its assumed random movement, will not navigate as a group through a transparent maze offered within the channel (see 2.5.1.) in which a strong light gradient has been set up (Figure 2, a deviant experimental setup from the unobstructed channel design, Fig. 1).

Our working hypothesis was that *Artemia* nauplii can follow the light gradient and thus navigate around obstacles through which a steady beam of unidirectional light is penetrating assuming that small differences in light intensity can be sensed by the *Artemia* nauplii. An additional expectation was that nauplii swarms should be sensitive enough to follow the most distance-efficient pathway when encountering the one obstacle that offers two alternative pathways (closest to the light in B, center in C and D, see Figure 2).

Hardware

The channel-mazes are basically an advanced channel design, now with added obstacles. The obstacles are either transparent coverslips made from thin transparent plastic or alternatively the same coverslips that partially covered with black electrical tape. As a result, the light intensity is suppressed and the light penetrates through two separate pathways (see Figure 2).

Procedure and statistical analysis

The same standardized density of *Artemia* nauplii again was placed at the bottom of the first barrier which was always positioned at base of the first obstacle in all treatments (Figure 2). The other two obstacles are varied in position creating alternative maze varients by placing them in the two possible places second or third one obstacle that offers two alternative gaps pass it, either in position 2 or in position 3 (see Figure 2). Therefore, *Artemia* are offered two alternative pathways in the transparent maze treatment. The maze with the partially opaque obstacles was only offered in one of the two alternative arrangements (see Figure 2).

At each location that offers an opportunity to pass a barrier through a gap, *Artemia* have been counted in ten replicated counting periods for a total standardized accumulated period of 60 seconds. The counting was strictly done at the edges of the barriers. Thus counts truly represent one-way movements with no chance to count the same individual twice at a given obstacle. The various counts have been used to analyze if 1. the swarm actually finds a path to the light, 2. if this path is also the most distance efficient path, and 3. If the *Artemia* find their way to the light source through a partially opaque maze. All together, we performed 9 maze experiments, using three types of mazes in three independent-brood replicates from three independently cultured broods. Aside from evaluating if *Artemia* actuall swarmed and reached the light source, we used mean values of passing *Artemia* at each barrier to get an idea about the flow through, and we compared mean numbers of *Artemia* passing at the obstacle offering two pathways, to test (student T-test, after F-test) if Artemia takes the most efficient path (see table 3).

2.5.2. *Light gradients along an elongated barrier*

The experimental set up consisted of a plastic petri dish entirely covered in black electrical tape except for openings on the left side which in this experiment were only used for illumination at three of five possible equivalent clock positions: 09:00, 10:00, and 11.00 (Figure 3). Attached to the center of the bottom-rim and to the flat-bottom of the petri dish a glass microscope-slide divides the petri dish into identical halves. This barrier reaches from the 06:00 position towards the 12:00 position, however, the slide is shorter than the diameter of the dish so that a small gap is left between the upper rim of the barrier and the top of the petri dish (Fig. 3).

Procedure

Two alternative treatments where performed. One treatment involves all alternative light positions, 09:00. 10:00, and 11:00. The other treatment involves only positions 9:00 and 10:00. In treatment 1 the light source is consecutively placed from 09:00 to 10.00 and from 10:00 to 11:00 with 5 minutes in between switches (Figure 3 short upper arrow). The result is that the lighted area expands towards the gap, because the angle at 10:00 illuminates the barrier at a larger angle than from position 9:00 and at 11:00 light illuminates the barrier at a larger angle than at positions 9:00 and 10:00.

In treatment 2, the light is moved from 9:00 to 10:00 after five minutes, but then not moved to position 11:00, it remains at the 10:00 position.

No light is offered in the lower half of the barrier. At the start, *Artemia* are placed right at the bottom of the barrier (see Figure 3, pink arrow). The only pathway around the barrier is located at the top of the barrier, a gap between the upper edge of the slide and the upper rim of the petri dish. Once *Artemia* pass over the edge of the slide and through the gap, they have a barrier-free path towards the actual light source at position 11:00 in treatment 1 (Figure 3 short upper arrow) and at position 10:00 in treatment 2 (Figure 3 long bottom arrow). We estimated the amount of barrier illuminated from the three alternative light position as follows: positions 60% of the barrier from position 09:00, which is less than 75% from 10:00, and 87% from 11:00. Thus, moving the light source in treatment 1 resulted in a stepwise extension of the illuminated light gradient nearer towards the gap than in treatment 2.

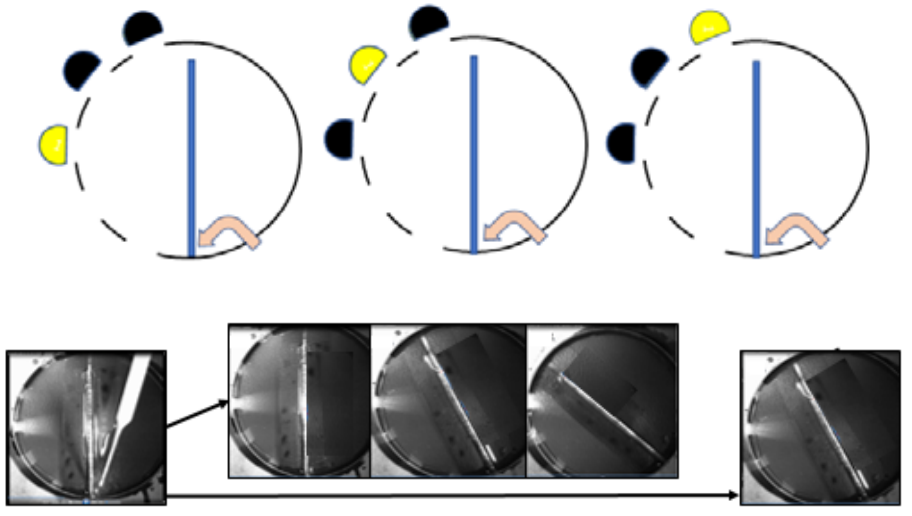

Figure 3: Standardized amounts of *A. franciscana* nauplii are placed at the bottom of the barrier (pink arrow) and the light (stereo microscope light) is focused through the gap at the 9:00 position onto the barrier which constitutes a position just above the midpoint of the barrier. Other light positions are established by rotating the dish to permit the light through the alternative openings (see bottom images). We performed three experiments (see shorter arrow) using three independent broods. Alternatively, a forth brood had been used to rotate the petri dish only from position 9:00 to 10:00 but then in remained at position 10:00 and no move to 11:00 was performed, a procedure that we call „control" (see Figure 3, the lower longer arrow).

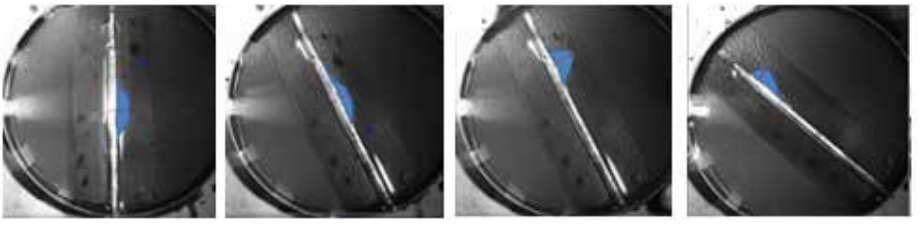

Figure 4: Visual examples to illustrate how the movement of the swarm (blue) was quantified in distance moved per minute (speed). The red line through the swarm is the swarm-axis line (from the highest point of the swarm to the intercept with the barrier line at a right angle).

Tracking the swarm

In order to quantify the movement of the *Artemia* nauplii from the entry point to the gap, movements were measured in centimeters along the barrier. To measure this distance, at each light position (9:00, 10:00. 11:00), the swarm's highest peak was located and an intercept of a line from the from the peak perpendicular to the barrier (Figure 4). The distance the swarm had moved was the distance from the bottom of the barrier to the intercept line. This way we were able to account for the changes in distance per time for each swarm in all treatments. The maximum time it took the swarm to reach the gap varied between 14 and 17 minutes, the time when the swarm completely dissolved and all swarming individuals had passed the barrier's upper edge and accumulated at the 11:00 light source (Figure 4).

Figure 5: Three types of obstacle channel designs. The the obstacle maze "B" and "C" feature unobstracted clear obstacles. Obstacle maze "D" has obstacles that are partially opaque.

3. Results

Three experiments have been carried out to investigate the swarming behavior of *Artemia franciscana* nauplii within horizontal shallow environments which were set up with strong light gradients.

First experiment: the most basic experiment investigated if horizontal unidirectional light-gradients and two-directional light gradients (point light from opposite sites) trigger similar responses, group formation, and motile behavior in *Artemia* nauplii. Repeated one-directional light-gradients and repeated reversals thereof are expected to result in repeated stable (non-significantly different) group patterns (Tables 1 and 2, Figures 1).

Second experiment: experiments investigate the behaviour of *Artemia nauplii* swarms within unidirectional light-gradients within horizontal pathways that offer transparent and also opaque obstacles. We used three obstacles within each maze but varied the arrangements within the same basic horizontal channel (Figures 3, 4, and 5).

Third experiment: a circular dish offering one elongated transparent barrier placed in the center dividing it into two halves leaving one small throu-way at the top only. *Artemia* entry point and throu-way were equally distant from a single light source located perpendicular to the barrier which was moved to two alternative positions at agreed times, resulting in light illuminating the barrier from different angles, setting up a progressing gradient of light over time (Figures 4, 6, nand 8).

Unobstructed channel – first experiment

We observed that repeated direction changes of single-light gradients do result in stable repeated re-organization of *A. franciscana* nauplii into organized swarms that move with the light gradient direction in similar ways and across consecutive trials. During repeated light direction reversals nauplii aggregations promptly reversed swarming direction maintaining similar passing rates at defined loctions (Fig. 1). The passing rates were significantly different comparing moves towards vs. away from the light source, and no significantly differences have been found across trials with the same light intensities offered from the same directions (left or right). When identical lights were used from opposite sites, the passing rates were also consistent across repeated trials but the passing rates towards the light and away from the light were not significantly different. This was true across multiple moves of the same swarms, across three independent repeated swarms from three independent broods (Table 1 and 2, Figure 1).

Table 1: F-/T-tests on nauplii counts in two-light treatments (2LT see Fig. 1).

brood 1	2LT	F-Test			t-Test: Two-Sample			
LEFT	LEFT							
to LT	**away LT**		*to LT*	*away LT*			*to LT*	*away LT*
4.1	9.2	Mean	6.83333	9.6667	Mean		6.83333	9.66667
10.4	12.7	Variance	10.4433	8.0033	Variance		10.4433	8.00333
6	7.1	Observations	3	3	Observations		3	3
		df	2	2	Pooled Variance		9.22333	
					Hypothesized	Mean		
		F	1.30487		Difference		0	
		p-value	**0.43386**		df		4	
							-	
		F Critical	19		t Stat		1.14261	
					p-value		**0.31695**	
					t Crit 2-tail		2.77645	
brood 2	2LT							
LEFT	LEFT							
to LT	**away LT**		*to LT*	*away LT*			*to LT*	*away LT*
4	7.8	Mean	6.7	9.3	Mean		6.7	9.3
10.7	13.3	Variance	12.49	12.25	Variance		12.49	12.25
5.4	6.8	Observations	3	3	Observations			3
		df	2	2	Pooled Variance		12.37	
					Hypothesized	Mean		
		F	1.01959		Difference		0	
		p value	**0.49515**		df		4	
							-	
		F Critical	19		t Stat		0.90539	
					p-value		**0.41646**	
					t Crit 2-tail		2.77645	
brood 2	2LT							
LEFT	LEFT							
to LT	**away LT**		*to LT*	*away LT*			*to LT*	*away LT*
6	7.2	Mean	8.5	6.4	Mean		8.5	6.4
13.3	7.6	Variance	17.29	3.04	Variance		17.29	3.04
6.2	4.4	Observations	3	3	Observations		3	3
		df	2	2	Pooled Variance		10.165	
					Hypothesized	Mean		
		F	5.6875		Difference		0	
		p value	**0.14953**		df		4	
		F Critical	19		t Stat		0.8067	
					p-value		**0.46507**	
					t Crit 2-tail		2.77645	

Table 2: Summary of the above t-test results from the unobstruted channel experiment. Brood (BR) 1,2,3 with 4 (single light, 1LT) or 3 (two lights, 2LTS) repeated treatments (REP) per brood. Bold numbers indicate that the mean number of *Artemia* moving towards the light was higher, regular numbers indicate that the mean number of nauplii moving away from light was higher, grey-background indicates non-significant differences between numbers of *Artemia* moving in opposite directions.

BR	REP	2 LTS Left to LT	2 LTS Left away LT	2 LTS Right to LT	2 LTS Right away LT	1 LT Left to LT	1 LT Left away LT	1 LT Right to LT	1 LT Right away LT
1	1	4.1	9.2	6.3	9.2	**14.1**	**9.7**	**16**	**12.3**
1	2	10.4	12.7	**15**	**10.6**	**18**	**13.1**	**16.4**	**11.7**
1	3	6	7.1	7.4	9.1	**16**	**8.3**	**17.6**	**11.9**
1	4	NA	NA	NA	NA	**22.3**	**10.3**	**20.3**	**7.5**
2	1	4	7.8	6.7	10	**14.4**	**9.7**	**12.2**	**6.6**
2	2	10.7	13.3	**14.7**	**10.3**	**12.4**	**6.6**	**15.8**	**10.6**
2	3	5.4	6.8	6.4	8	**16.3**	**8.6**	**16.3**	**11.8**
2	4	NA	NA	NA	NA	**16.3**	**8.6**	**16.3**	**11.8**
3	1	6	7.2	8.7	10	**12.6**	**9.7**	**15.6**	**12.1**
3	2	13.3	7.6	15.1	10.7	**13.9**	**9.4**	**1.1**	**6.2**
3	3	**6.2**	**4.4**	9.9	7.1	**15.1**	**10.7**	**18.6**	**12.9**
3	4	NA	NA	NA	NA	**16.2**	**12.7**	**17.4**	**10.1**

Obstacle channel experiment – second experiment

In the various maze designs using transparent obstacles in the channels, *A. franciscana* nauplii swarms found their way from the starting point to the light source, however the paths taken were not length-optimized: *Artemia* did not chose the shortest (most economic) overall pathway (Tables 3, Figure 5). In channels with partially opaque obstacles, the swarm basically disintegrated after passing the second barrier. The opaque parts of each barrier seem to obstruct the light gradient in a way that the nauplii act individually, some make it all the way to the light source.

No organized swarm or any kind of non-random movement was observed without any light gradient, neither in the straight channel nor in the circular dish design.

Table 3: Number of *Artemia* nauplii passing the edges of three obstacle (OBS) (see Figures 2 and 5)

Obstacle maze „B“ clear	**Brood 1**	**Brood 2**	**Brood 3**
OBS1 TOP	114	196	221
OBS2BOTTOM	107	134	124
OBS3BOTTOM	**57**	**59**	**58**
OBS3 TOP	**95**	**56**	**36**
		Two-path obstacle (OBS3)	
		bottom	top
		57	95
		59	56
		58	36
Obstacle maze „C“ clear	**Brood 1**	**Brood 2**	**Brood 3**
OBS1 TOP	119	177	200
OBS2 BOTTOM	**111**	**169**	**112**
OBS2 TOP	**101**	**80**	**60**
OBS3 BOTTOM	73	61	38
		Two-path obstacle (OBS2)	
		bottom	top
		111	101
		169	80
		112	60
Obstacle maze „D“ cover	**Brood 1**	**Brood 2**	**Brood 3**
OBS1 TOP	80	69	91
OBS2 BOTTOM	**0**	**0**	**2**
OBS2 TOP	**31**	**25**	**43**
OBS3 BOTTOM	0	0	1
		Two-path obstacle (OBS2)	
		bottom	top
		0	31
		0	25
		2	43

Long barriers within continuous light gradients – experiment 3

Upon adding *Artemia* at the bottom of the barrier (Figure 6, top, see arrows) the nauplii immediately moved within the most direct light impact positioned at 09:00 forming a well-defined swarm (Figure 6 bottom) within a minute. When the light was moved to position 10:00 the swarm moved quickly to the equivalent location at the barrier, and did so again following the move of the light source to position 11:00 (Figure 6 bottom) and further to the edge of the barrier. From there *Artemia* quickly passed the edge of the barrier and accumulated as very dense swarm at the actual 11:00 light source location.

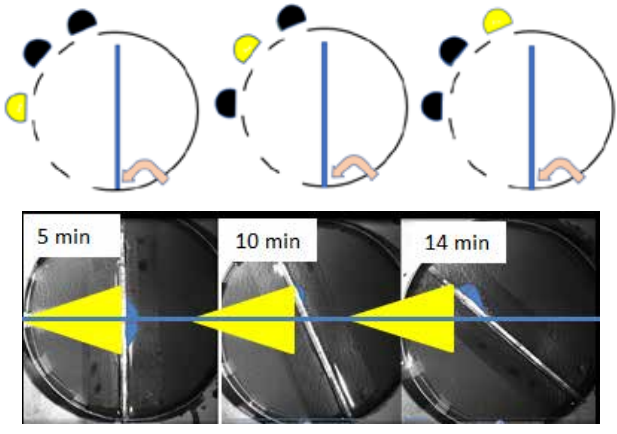

Figure 6: Change in illumination as the light is moved from position 9:00 to 10:00 to 11:00. *Artemia* nauplii follow a stepwise-maintained gradient of light.

In the maze (experiment 2), *Artemia* formed an organized swarm at the first obstacle but gradually became less organized at the 2nd and 3rd obstacle. In contrast, the swarm in experiment 3 started as a very tightly organized swarm and continued to move as that following the entire length of the barrier. Nevertheless, some individual *Artemia* did leave the swarm, however, they continued to move along the barrier The overwhelming majority of *Artemia* stayed within the swarm context. Only at position 11:00 at the barrier did the swarm quickly dissolve and individuals moved around the barrier's edge an on towards the actual light source (Table 4).

Table 4: Distances and total time *A. franciscana* nauplii moved as a well-defined swarm along the central barrier (see Figure 6 and 7)

time (min)	brood 1 1st, 2nd, 3rd light	brood 2 1st, 2nd, 3rd light	brood 3 1st, 2nd, 3rd light	control only 1st, 2nd light
1	6	6.4	6.1	6.1
2	6.4	6.5	6.6	6.9
3	7.2	7	6.9	6.9
6	7.2	6.9	7.6	7.2
7	8.5	8	9	9.4
8	10	9.9	10.2	10.4
9	9.9	10	10.4	10.1
10	10.3	10.1	10.5	10.3
13	12	10.6	12.6	10.4
14	13	12	12.7	10.6

During the move of the swarm we recognized phases of fast movement immediately following the placement of the light source at the next position, which was followed by swarm stagnation at the current light position until another move of the light source was executed (Table1, Figure 7). No *Artemia* moved into any other direction but along the barrier, thus all Artemia, those swarm-bound and those individually moving stayed within the continuous light gradient.

In summary, we observed obvious differences in *Artemia* swarm-organization within a maze vs. along a continuous barrier. In the maze it is difficult to predict the light gradient. *Artemia* were not able to distinguish between pathways and dead-ends and were not able to choose the more efficient path at obstacles with two alternative pathways. Within a maze consisting of separate and spatially independent barriers swarms tend to disintegrate into smaller groups. In contrast, the swarm moving along a single barrier and within a continuous and highly predictable light gradient stayed intact.

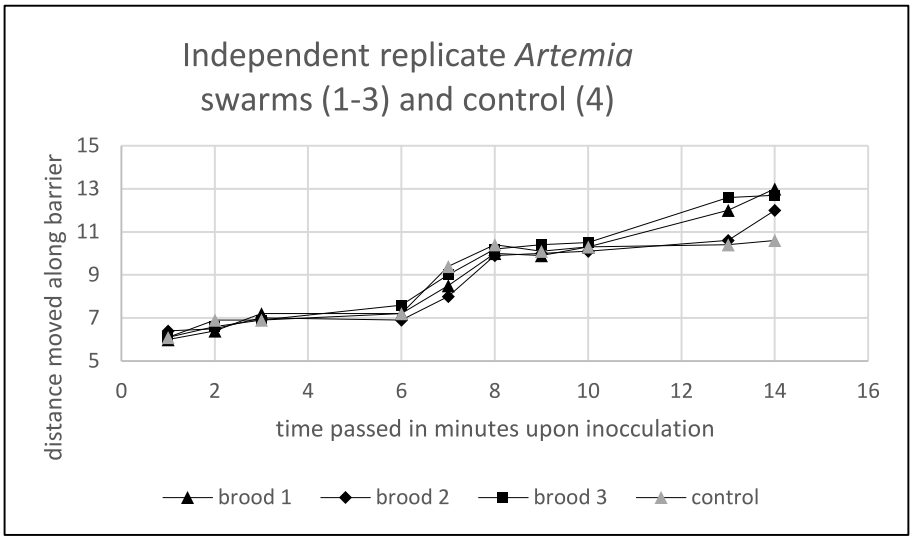

Figure 7: Movement of *A. franciscana* nauplii swarms in response to light switches from 1st to 2nd to 3rd light position

Even though when the swarm ("control 4") in experiment 3 was arrested at position 10:00, individual *Artemia* continued to move along the barrier and towards the edge of the barrier (Figure 7).

Discussion

Experiment 1: In response to switching the directional light conditions from the left to the right and vice versa, *Artemia* nauplii showed *non-random movement, significantly more nauplii moved towards the light than away from the light*. In contrast, we observed non-signifcant differences in the passing-rates of *Artemia* nauplii at each of the two simultaneous lights at opposite sides, left vs. right. *We must assume random movements.* The first experiment confirms that directional light results in directional swarming while non directional light causes random movement. This is in line with other studies that also applied directional vs. uniform light in a vertical set-up [16]

The nauplii swarm quickly responding to changes in light conditions, when unidirectional light is switched from the left to the right and vice versa they move with comparable speed into the new direction, and reverse direction immediately. Likewise a switch from the unidirectional light to a two-directional light arrangement quickly becomes random

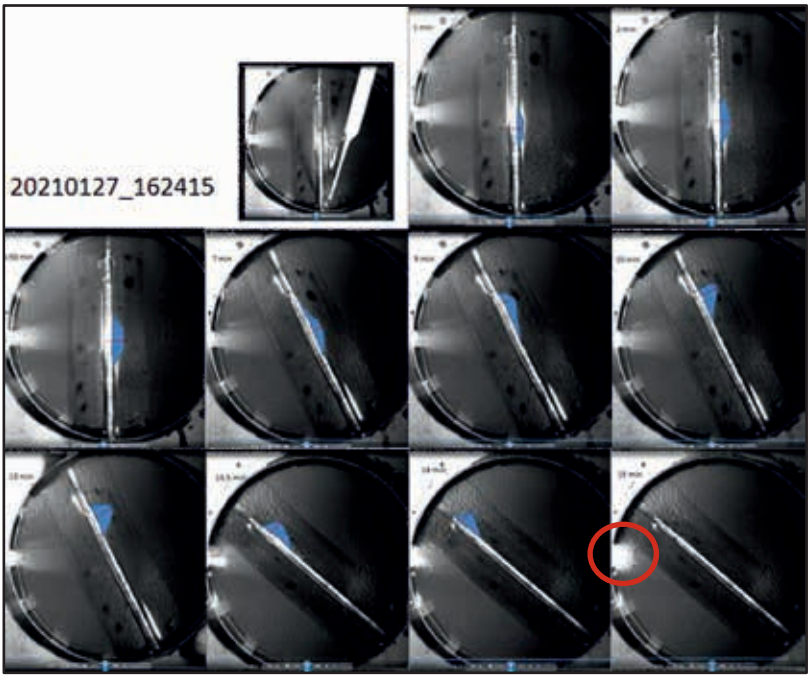

Figure 8: Petridish with three entry points of a standardized light source. *Artemia nauplii* are placed at the bottom right of the barrier, and the swarm forms and moves with the light gradient along the glass barrier to the light in position 9:00 (phase 1, 0-5 min), followed by phase 2 (5 -10:00 min) upon a move of the light source to position 10:00 and further to position 3 (11:00). The swarm moves with the changing light position along the barrier until all individuals within the swarm moved all the way to the far end of the barrier, spilling over the edge, and accumulate at the actual light source. After a total of 14-15 minutes, all *Artemia* form a tight swarm around the actual light source (see red circle around a bright glowing swarm). The swarm itself seems to „slow down"at every light position and „pauses in place" until the light source is moved to the next position. In the "control" experiment, we did not move the light source from position 10:00 to position 11:00, the swarm remained at the 10 min light source position, but individual *Artemia* nauplii did move along the barrier and around the barrier however, a few more minutes passed before all *Artemia* had gathered again as a swarm at the light at position 10:00, approximately after the 17th minute.

around each of the two local light, yet light maintained an associated local swarm with a steam of Artemia moving between the swarms. This pattern supports observations in nature when large swarms form vertically or horizontally aggregates which are composed of several denser aggregations thinly connected with each other by thin

bands of commuting individuals [1].

The fact that Instar I nauplii at that developmental stage are supported by yolk food may support the extremely enduring activity level observed. When nauplii hatch from a metabolically inactive cyst-eggs, they have dormant rested in dried-up mud, or salt-crystal crusts, or in sand mixed with rocks, etc... all dried up for lack of precipitation. When precipitation returns, and water accumulates, hatching is the response

The cysts hydrates and the arrested embryo resumes to metabolize. Two days later, the cyst's membrane breaks open and an embryo appears which needs a few hours more to develop into the instar I nauplius. It will immediately be capable of free swimming. It is equipped with a red nauplius eye in the head region and it has three pairs of appendages (sensory antennae and locomotive antenna) but the instar I nauplii do not immediately take up food, the digestive system is not functional yet. It lives off its yolk reserves. While its appendages further develop into soon-needed plankton-filters and food-ingestion tools the most important step to survive is to seek the surface, where plankton resides. With the yolk sack still there the young *Artemia* is negatively buoyant and will have to actively swim there. If upon hatching they encounter a homogeneous water body they will sense the direction to the surface easily, their single eye guides them and they are good swimmers. If there are other nauplii around, it is beneficial to join into groups as movement is energetically more efficient in a group, especially for small animals. Aside from following the light they will align with others, looking out to avoid collisions. A vertical swarm forms.

Once they reach the surface they should maintain this position and start being preoccupied with sensing particles at the surface, possibly something they can see with their eyes, maybe by recognizing promising spectra as a proxy. Horizontal movement could be important now to move between food rich patches and once there, resume vertical swarming.

Vertical swarming potentially supports efficient feeding. From my casual observations of the patterns within the swarms, I noticed that within swarms some kind of convections cells form. Some nauplii move to the light thus surface and others volunteer to move away from the light and thus downwards. The whole pattern resembles a close loop conveyer-belt. Food particles could be collected that way and passed through the swarm but also be moved from the outside of the swarm into the swarm. A great benefit would also be the circulation of water from the outside into the swarm to replenish oxygen.

Experiment 2: The second experiment showed that *Artemia* nauplii are capable of following obstructed light gradients within a straight channel if the obstacles are transparent but not when the obstacles are opaque, thus blocking the light and creating patterns of shade. We also showed in experiments 2 that nauplii have no sense for very small differences in light. Nevertheless, all *Artemia* that, in experiment 2, passed the first obstacle in the transparent maze arrived eventually at the light source. The question

again is how does a within-a-maze behavior in the lab reflect genetically based behavior of nauplii in their natural environment? Is there a maze-challenge at the edge of dry ponds or lakes in the process of filling up with water? There is.

When a Salt Lake or a commercial salt pond dries up, the surface is a tangle of salt crystals, mud, small rocks. The cysts that develop into a hatching Instar I nauplius reside in a maze of structures, now increasingly covered with rain water or flood water. What are the environmental cues to guide the hatching nauplii? Again there is a light gradient to follow, but not a very uniform one. First the nauplii need to navigate through the debris and the salt crystals. As experiment 2 has shown, this navigation is not a simple swarm path. Instead groups of nauplii may move along each obstacle but each individual must make local decision to maintain an overall path to the light, to the water's surface. Within this obstacle maze they join and leave various smaller aggregations. Eventually most of them will find their way out and reach the open water. This natural maze is a place of scattering, reflection, shading and darkness, so we would not expect dense uniformly moving swarms to follow it. It has been shown that individual nauplii can be attracted to each other part-time and at random, joining and leaving groups [17].

Experiment 3 has shown that nauplii will master a barrier within a coherent swarm as long as there is a clear light gradient that is maintained. The resulting movement is very efficient, the swarm moves as a unit and fast. However, we have also seen that the loss of the light gradient does not mean that nauplii just move totally randomly in all direction. While individuals do move out of the swarm, they eventually find the way in the correct direction. In experiment 3 they all found the gap followed by a very direct swift swim to the light source. All *Artemia* ended up at the light source it just took a bit longer to get there.

In their natural environment feeding at the surface *Artemia* can be exposed to dangerously high levels of radiation. As scattered as natural *Artemia* populations are across the planet, their preferred lakes and ponds are in areas with intense solar radiation. Deadly UV-B must be avoided. Joining dense horizontal swarms may be beneficial again. Light can be an attraction and repulsion. UV-B are not directly sensed, but UVA levels are proportional to the intensity of UV-B. Measuring UVA is a proxy for truly damaging radiation that has been discovered in many terrestrial and marine organisms from phototrophic bacteria [18] to eukaryotic algae [19] and planktonic larger animals [20].

4. Conclusions

Much more work could be invested in understanding *Artemia* behaviour in their natural environments or in artificial salt ponds. Studying the indoor models will instruct us about the potential and the variety in swarming, grouping, and individual behaviors. Likewise studying them in the lab will help us to improve *Artemia* production for a

sustainable aquaculture with a circular feed-production of Artemia and for enhancing bio-incapsulating methods used to transfer medications and supplements directly into the target species body rather than distributing these substances in the surrounding water from where they easily may move into natural habitats, potentially doing harm. *Artemia* are definitely worth more research in both: basic ecology and evolution studies and in applied research alike.

We are planning to further analyze our data collected in the three experiments described here. The follow up analysis is to look at individual's movement within the experimental treatments. What are the algorithm behind the movements and behaviors? What are the rules for swarming, for random grouping or for individual actions in Artemia?

5. Acknowledgements

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