

**Video Analysis and Documentation of Eusocial Behavior in Commensal Amphipod
Crustaceans**

Honors Thesis

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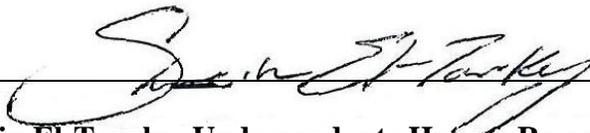
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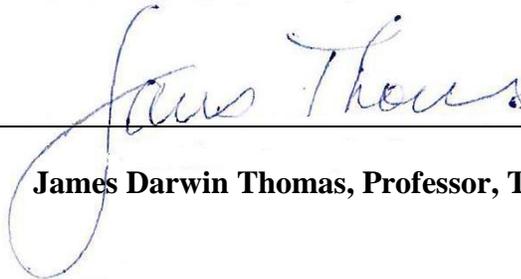
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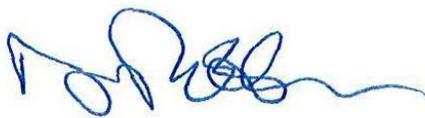
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<u>Table Of Contents</u>	Page (s)
I. Section I: Abstract.....	4
II. Section II: Objectives.....	5
III. Section III: Introduction	
A. Eusociality	7
B. Eusociality in Marine Organisms.....	7-8
C. Superorder Peracarida.....	8-9
D. Order Amphipoda.....	9
E. Niche Specificity in Order Amphipoda.....	9-10
F. Family Leucothoidae.....	10-11
G. Anamixis Clade.....	11-12
H. Organisms Selected for Study.....	12
IV. Section IV: Materials and Methods.....	13 -14
V. Section V: Results.....	14 -21
VI. Section VI: Discussion and Conclusion.....	21 -23
VII. Section VII : References	
A. Literature	24-25
B. List of Figures	
A. Figure 1.....	26
B. Figure 2.....	26
C. Figure 3.....	26
D. Figure 4.....	26
E. Figure 5.....	26
F. Figure 6.....	26
G. Figure 7.....	26
H. Figure 8.....	26

Table of Contents

Page (s)

VIII. Section VIII: Acknowledgements.....27

IX. Section IX: Appendix 1

A. Video Capture from Camera.....28
B. Video Capture from Mini-Dv Player.....28-29
C. Basic Video Editing.....29
D. Video Output.....29
E. DVD Burn.....30
F. Camera To Monitor.....30
G. Computer to Monitor.....30
H. VCR To Monitor.....30
I. Mini- Dv Player to Monitor.....31

I. Abstract

Eusociality is a term used in reference to the highest level of social organization in a hierarchical classification of populations and is typically associated with terrestrial invertebrates such as ants and bees. A species is typically deemed to have eusocial organization when it exhibits several complex social behaviors including cooperative care of young, nest guarding, and reproductive division of labor. Recently, scientists such as Emmet Duffy have documented eusociality in marine crustaceans like the snapping shrimp, *Synalpheus filidigitus*. It is known that Gammeridian Amphipods have a social structure, but a determination as to whether or not their behavior is eusocial, *sensu-stricto*, remains to be determined.

Specimens of Gammeridian Amphipods residing commensally within transparent tunicates, *Ecteinascidia turbinata*, were collected *in situ* from the Florida Keys. The amphipods were filmed within the tunicates in microaquaria by special video cameras to record and document all behaviors, interactions, and any recognizable fixed action responses for later analysis and classification. The resulting behavior profile revealed that the commensal amphipod under study, *Anamixis cavatura* Thomas, 1997, exhibits several complex behaviors and fixed action responses as well as recognition of “kin”. The documented behavior profiles provide a strong basis for further investigation into possible mechanisms of eusocial structure in these amphipods.

II. OBJECTIVES

- To locate and obtain specimens suitable for filming and analysis.
- The development of unique techniques designed for filming through both microscopes and marine micro-aquaria,
- To film and observe in-situ behavior of *Anamixis cavatura* and interpret the behaviors and actions of the specimens.
- To utilize and further develop superior imaging techniques through the use of z-stacking software. *

* Not an intended consequence but a subsequent outcome of the project.

III. Introduction

A. Eusociality

Eusociality is a rare evolutionarily behavior that is used only in reference to the highest level of social organization in a hierarchical classification. It has previously been observed only in the insect orders Hymenoptera, and Isoptera, and in mammalian mole rats. A species is typically deemed eusocial when it exhibits several complex social behaviors including cooperative care of young, nest guarding, and reproductive division of labor.

The first studies conducted with respect to eusociality were completed in order to determine whether eusociality is a behavioral adaptation that enables organisms to increase their fitness and reproductive success (Wilson 2005). In ants, kin selection and eusociality have been pinpointed as the key to their ecological dominance and it is believed to have even influenced ant phylogeny (Wilson 2005). However, Charles Darwin, in his work *The Origin of Species*, noted that eusocial behavior was inconsistent with Darwin's assertion that organisms strive to increase their own fitness and reproductive success. This contradiction remained unresolved until 1964 when William Hamilton developed a model to explain this behavior. In his model, which he named kin-selection, he demonstrated that eusocial organisms are more closely related, genetically, than non-eusocial organisms (Poulton, 2002). Furthermore, by assisting in the rearing of siblings, eusocial organisms are increasing the likelihood that their genetic traits will be manifested in future generations.

B. Eusociality in Marine organisms

Observations of eusocial behavior amongst marine crustaceans have only recently been made. The primary author of literature concerning eusocial behavior amongst marine crustaceans is Dr. Emmett Duffy. In 2001, Dr. Duffy described the eusociality of *Synalpheus regalis*. He found that the social structure of *S. regalis* included a large, breeding female termed the queen. He also found that a differentiation of tasks based on gender and relative size also existed amongst Amphipoda. Overall, he discovered that this tiered social hierarchy was useful in colonial defense, breeding, and increasing the overall fitness of the organisms. Contributions on the discovery of eusocial behavior in gammaridean amphipods was documented and described by Thomas (1997).

C. Superorder Peracarida

The Crustacean Superorder Peracarida consists of roughly 13,000 species, approximately 8,000 of which belong to the Order Amphipoda. Like all peracardians, amphipods possess only one true thoracic segment, bear eggs in a thoracic brood pouch, and lack a true larval stage. The characteristics that distinguish Amphipods from other peracardians include a laterally compressed body with ventrally oriented coaxal plates, sessile eyes, the absence of a carapace, lack of antennal squame, and seven pairs of uniramous pereoneal walking



Figure 1- Tropical, Pacific Amphipod.
Thomas and Klebba, 2009.

limbs (Bousfield 1973). The first two appendages are specialized for feeding as pincers. An important morphological trait of the amphipod is their distinct gills, called coxal or thoracic, which are located on the walking limbs. Amphipods are segregated into four suborders; Gammaridea, Hyperiidea, Ingolfiellidae, and Caprellidae.

D. Order Amphipoda

Of the more than 8,000 named species, the majority of Amphipods are marine denizens although, there are limnic and terrestrial representatives within the order. Amphipods have adapted to a variety of environments and oceanic zones. Consequently, benthic and pelagic representatives have been found in a variety of locations including coastal, shelf, slope, and hydrothermal vent communities in both in both epifaunal and infaunal niches. Often the most abundant organisms present in these various habitats, amphipods represent a significant part of the Trophic Cycle. Amphipods also play varied roles within this cycle as herbivores, detritivores, micropredators and scavengers (Lowry 2001).

E. Niche Specificity in Order Amphipoda

Though Amphipoda inhabit a wide



Figure 2-Ectocommensal Amphipods, Thomas and Klebba, 2009

Figure 3- Endocommensal Amphipod Thomas, 2009.

variety of environments, families
Figure 2- Ectocommensal Amphipod Colony ty and
Thomas, 2009.

are often found in conjunction a host that provides and maintains the conditions required by the organism. Thus, the many families of Amphipods are commensal, selecting hosts such as sponges (see below) or tunicates (see right) and associating with them either ectocommensally or endocommensally. The Family Leucothoidae, for instance, exhibits this type of ecological interaction. Endocommensal organisms, such as *Anamixis Cavatura*, are those that reside within an organism while ectocommensals populate the outside of an organism.



Figure 4- Leucothoid Amphipod 1
Thomas and Klebba, 2009.

F. Family Leucothoidae

Members of the Family Leucothoidae, under the suborder Gammaridea, were among the first amphipods to be described. They were initially described in the

Skagerrak Sea, Denmark by Abilgaard (1789). The structure of the family, however, was not established until 1814 (Leach). “The Family Leucothoidae currently contains 138 species in six genera: *Anamixis* Stebbing, 1897 (21 spp.);



Nepanamixis Thomas, 1997a (4 spp.);

Figure 5- Leucothoid Amphipod 2. 2009

Paranamixis Schellenberg, 1938 (13 spp.); *Leucothoe* Leach, 1814a (96 spp.); *Leucothoella* Schellenberg, 1928b (2 spp.); and *Paraleucothoe* Stebbing, 1899 (2 spp.)” (Thomas 2009). Leucothoidae is subdivided into two primary clades; *Leucothoe* and *Anamixis*. Leucothoids can be found distributed throughout both polar and equatorial waters while anamixids are relegated to primarily tropical and warm-temperate reefs.

G. Anamixis Clade

The anamixid clade, 33 species, is also much smaller than the Leucothoid clade which consists of around 92 species. Recently, the two clades were combined (Lowry *et al.* 2000). This was a controversial taxonomic change due to the apparent dichotomy between the strong sexual dimorphism found in the anamixid clade as opposed to the slight dimorphism found in the Leucothoid

clade. In anamixids, the dimorphism is so distinct that males and females were

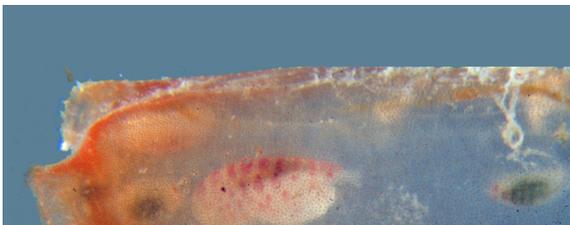


Figure 6- Anamorph and Leucomorph in Tunicate. Thomas and Klebba, 2009.

classified into separate families. However, “In 1983 Thomas & Barnard announced the transformation of males of *Leucothoides pottsi* (Leucothoidae, Dana, 1852) into hyper adult males of *Anamixis hanseni* (Anamixidae Stebbing, 1897; [now *Anamixis cavatura*, (Thomas, 1997a)]” (Thomas 2009). Thus, in a single-molt leucomorph males were observed to transform into anamorphs- hyper adult males- previously described as anamixids. It is unclear as to how many other taxa are also affected by this discovery.

H. Organisms Selected for Study

Anamixis cavatura was selected to be the target organism of this study based on several variables. Due to evidence of eusociality found in marine crustaceans, first by Dr. Duffy and later by Dr. Thomas, it is likely that this behavior will be exhibited by other members of this subphylum. In addition, *Anamixis cavatura* is a species that is majorly undocumented and, thus, potentially informative as little is known about the intra-colony and inter-colony interactions. Ease of specimen acquisition



Figure 7- *Anamixis cavatura* on *Ecteinascidia turbinata*. 2009.

was also a major concern due to the time frame of this project. Thus the habitat of this organism, which is in shallow water in many readily accessible locations, was also an integral factor in the selection of *Anamixis cavatura* as the target organism. Finally, the affinity of *Anamixis* for habitation of the transparent and

prolific tunicate *Ecteinascidia turbinata* was also conducive to the collection of specimens as well as *in-situ* filming.

IV. Materials and Methods

Specimens were collected *in-situ* from two primary locations. The first collection was acquired on September 4th, 2009 in the water surrounding Sea Camp on Big Pine Key. Specimens from the second collection were procured on September 25, 2009 from the Keys Marine Lab on Long Key. *Ecteinascidia turbinata*, a host tunicate often inhabited by *Anamixis cavatura*, were collected by hand while snorkeling from various substrates; mangrove roots, coral heads, and the Sea Camp Swimming Dock. Once the colony was detached from a surface, it was rapidly placed within a *Ziploc bag*. Once on land, Zooids containing amphipods were placed in separated Petri Dishes in order to keep colonies distinct. While being filmed, the zooid was either retained in the Petri dish or translocated to mini- aquaria. Mini- aquaria were designed by Dr. James Darwin Thomas by assembling slide covers in boxes of various areas and fixing them together with silicon. The base of the mini- aquaria was always a glass slide.

Filming through a Dissecting Microscope was done using a SONY DCR-TRV900 video camera with a M-99 adapter. For filming of organisms within micro-aquaria, a SONY DCR-TRV900 video camera with a 3 chip CCD and a Zeiss Objective lens was used. In both cases, the camera was attached directly to A SONY BRAVIA KDL-19M4000/B U2 monitor via an S-video cord. Filming at location 1 was done through a dissecting scope while zooids were placed in micro-aquaria for filming at Location 2. For filming at location 2, the micro-aquaria were placed on an adjustable stand that was

adjusted to the height of the camera which was also on an adjustable stand. A piece of dark velvet was used as a background.

All film acquired was stored on mini-DVR tapes. The tapes were inserted in to a GV-D1000 mini- DVR player which was connected to a laptop via a Firewire 1394 port. Files were stored on an external hard drive that was connected to the laptop. Film was imported from the mini-DVR player and translated into .avi files via Pinnacle Studio 12 import and editing software. Further editing of the collected film was done using Pinnacle Studio 12 software. See Appendix 1 for a summary of the instructions on how the various devices used within this experiment were connected.

V. Results

The best method for recording amphipod behavior was determined in a lab prior to specimen collections at locations 1 and 2. Two types of cameras were tested for potential use in filming through a microscope. Ultimately, it was determined that the SONY DCR-TRV900 would be the camera of choice due to its compatibility with the Pinnacle 12 Editing Software. The other camera tested was the *Lumenera Infinity 3* Microscope Camera. This camera proved more ideal for smaller objects and organisms that did not moves as quickly as the amphipods we were filming. In addition, the files acquired using this camera were difficult to manipulate with video editing software.

Both light and dark backgrounds were tested for use when filming the amphipods. It was determined that darker backgrounds are more ideal for the filming of the amphipods as the darkness of the backdrop contrasts more with both the tunicates and the amphipods. In some instances, however, the lighter background proved useful, especially in instances where two or more amphipods overlapped one another.

Two filming set-ups were tested in this experiment. At location 1, all filming was done through a microscope with the zooid being observed being located in a petri dish. This set-up presented several challenges. The primary complication was that any movement, however small, could cause the zooid to drift or spin and thus constant readjustments needed to be made which interrupted observation of amphipod behavior resulting in less usable film of behaviors and longer editing time of film. Benefits were that the whole zooid could be observed making it easier to follow large migrations of the amphipods. In addition, placing the zooid made it easier to adjust the position of the zooid when an amphipod moved out of focus. This type of filming was also ideal for observation of tunicate behavior and morphology including circulation and expulsion of fecal pellets.

In location 2, all filming was done in micro-aquaria. The advantage of this method was that there was little movement of the zooid and any readjustment was easily done by rotating the micro-aquaria. A disadvantage to this style of filming was that when animals exited the tunicate and entered the corners of the aquarium they were difficult to film. Lighting also proved a more difficult task in this second filming method. For both filming methods it was extremely important to avoid shaking or any other disruption of the filming set up.

Observations of the general ecology of *Anamixis caviura* were made during both collections. At location 1, defined as the sites surrounding Sea Camp, it was found that an average of 1 out of every 75 zooids contained amphipods. In each populated zooid, 1 to 2 anamorphs and 7 to 12 leucomorphs were observed. At location 2, defined as the sites located around the Keys Marine Lab, the number of populated zooids was significantly

smaller and the ratio of inhabited to uninhabited zooids was approximately 1 in every 150. In both sites, and in all the inhabited zooids observed, the activity level of leucomorphs was markedly greater than the activity level of the anamorphs. Anamorphs were observed primarily in the branchial basket of the tunicate. In several zooids, anamorphs were also observed in the excurrent canal, but anamorphs were never observed outside of the tunicate. Leucomorphs were observed in the branchial basket and excurrent canal as well as outside of the tunicate. In location 1, leucomorphs were even observed swimming away from the tunicate and returning later to the same tunicate.

Interactions between amphipods were observed closely. Within a single zooid, leucomorphs and anamorphs from the same zooid were not observed to interact. Leucomorphs were observed moving throughout the tunicate while anamorphs remained in the same spot, moving only their appendages or to adjust their position within the water current. Leucomorph to leucomorph interactions between animals from the same tunicate were minimal and leucomorphs appeared to only navigate around one another.

Observations were made of a leucomorph leaving a zooid and entering the branchial basket of a neighboring colony. The anamorph residing in the invaded tunicate responded aggressively. First, the anamorph moved from its position which, as noted before, is uncharacteristic. Then, the anamorph slowly advanced towards the intruding leucomorph while moving his head up and down. The leucomorph responded by retreating towards the mouth of the tunicate. The anamorph continued this aggressive behavior until the intruding leucomorph was on the lip of the tunicate. The intruding leucomorph attempted reenter the branchial basket three times, but the anamorph became agitated and ejected the leucomorph each time it wandered 2 or more millimeters into the

branchial basket. This behavior was not observed between anamorphs and leucomorphs of the same colony. The attempt of a foreign leucomorph to enter an inhabited zooid was only observed in location 1.

At location 2, two anamorphs from different colonies were put in the same micro-aquaria. Initially, both exhibited aggressive behavior including the head gyrations observed earlier. Resultantly, the more dominant pushed the weaker anamorph to the corner of the micro-aquaria. Then, both remained in the corner side by side facing opposing direction and exhibited feeding behavior.

The most common fixed-action responses observed in both Anamorphs and Leucomorphs were related to feeding. Feeding behavior, in leucomorphs, was observed both outside and inside the tunicate in both the branchial basket and the excurrent canals. Anamorphs were only observed feeding inside the tunicate, but they were noted in both the branchial basket and excurrent canals. One of the first feeding related responses observed was that the orientation of the organism was dependent on the direction of the water current. Amphipods always faced the current head on. Thus organisms in the excurrent canal were found to face the closed end of the tunicate while those in the basin were found to face the mouth of the tunicate. Orientation of leucomorph found outside of a tunicate was dependent on water flow. Leucomorphs, in water with no current, were observed to generate their own current using the motion of their pleopods, this is fixed-action response 14. Pleopod rates were determined by observing organisms in different orientations on or in the same tunicate. Average rates of organisms from the same tunicate were compared to one another. Pleopod motion was counted within a time interval of 5 seconds. Six 5 second intervals were averaged together to acquire the rate.

The average rate of pleopod movement within the tunicate was 2.5 beats/ second while the average rate outside of the tunicate was calculated to be 6 beats/ second.

Within the tunicate, the periods of pleopod rest were more frequent than in organisms outside of the tunicate. The frequency of stops and length of pauses calculated were semi-variable but the averages are listed in Table 1. The discrepancy in length of pause in pleopod motion between anamixids on the outside of the tunicate and those inside was significant.

Table 1- Observations of Pleopod Movement

Behavior	Location	Average Rate
	Inside Tunicate	2.5 beats/sec
Rate of Pleopod Movement		
	Outside of Tunicate	6 beats/sec
	Inside Tunicate	.27 stops/sec
Cessation of Pleopod Movement		
	Outside of Tunicate	.13 stops/sec
	Inside Tunicate	1.8 sec
Length of Time Pleopod Movement		
	Outside of Tunicate	6.7 sec

Anamixis cavatura gleans nutrients from the water by catching them on the microscopic setae located on various parts of their bodies. These nutrients are then collected by “cleaning” their bodies by brushing the body part with their gnathopods, which also have setae. The gnathopods then bring the nutrients back to the mouth where they can be consumed. There were four types of cleaning observed in *A. cavatura*. There was no difference between the feeding behaviors observed in anamorphs and leucomorphs other than location. Leucomorphs were observed feeding both inside the tunicate and outside of it. The most common cleaning behavior, fixed-action response 11,

observed was antennal cleaning which occurs when the organism bends one of its antennae towards its mouth until the mouthparts are able to comb the nutrients off the antennae. The second type of cleaning, fixed-action response 10, was the cleaning of the pleopods. For this behavior, the organism was observed to arch its back and brush the setae of its gnathopod 2 across the pleopods. A third form of cleaning exhibited by the anamixids was carapace cleaning. This cleaning occurred when the gnathopod 2 was swung backward over the dorsal side of the organism and the carapace was repeatedly brushed with the gnathopod 2 setae. The final form of cleaning observed was carpal lobe cleaning; the rubbing of the setael tufts, located on the carpal lobes, in opposing directions of each other. Following the scraping, the food is then brought up with the maxilliped palp to the mouth.

Table 2- Observed Behaviors

Behavior Number	Ascribed Name for Fixed Action Response	Description
1	Extra-Tunicate Movement of Leucomorph	Movement of leucomorph on the outside of “home” tunicate
2	Leucomorph Migration	Movement of leucomorph from one tunicate to another
3	Non-Kin Recognition	Observed only in anamorphs; anamorph-leucomorph interaction
4	Kin Recognition	Non-aggression between anamorphs and leucomorphs of the same colony
5	Current-Based Orientation	Positioning of organism based on direction of water flow
6	Branchial Basket Feeding	Feeding of amphipod within Brachial Basket of Tunicate
7	Excurrent Canal Feeding	Feeding of amphipod within Excurrent Canal of Tunicate
8	Extra-Tunicate Feeding	Feeding of amphipod on the outside of the host tunicate
9	Surface Tension Feeding	Feeding of amphipod within the surface tension of the water
10	Pleopod Cleaning	Use of gnathopod to clean Pleopods
11	Antennal Cleaning	Cleaning of Antennae via Maxiliped Palp
12	Carapace Cleaning	Amphipod cleans carapace using gnathopods
13	Carpal Lobe Cleaning	Amphipod cleans underside using pleopods and gnathopods
14	Generation of Feeding Current	Amphipod uses pleopods to generate current

Upon examination of the tunicate, the circadian heart rhythm could be observed. An important observation to note is the reversibility of the heart rhythm that can be induced by disruption of the tunicate, and which also occurs spontaneously.

Finally z-stacking and video editing software were used to take premium quality photographs. Photoshop Elements 8 was used to further

Figure 8 - Anamorph Edited



VI. Discussion and Conclusions

The objectives of this experiment were:

- To locate and obtain specimens suitable for filming and analysis
- The development of unique techniques designed for filming through both microscopes and marine micro-aquaria,
- To film and observe in-situ behavior of *Anamixis cavatura* and interpret the behaviors and actions of the specimens.

Indeed, the specimens required to conduct this study were located at both research sites, though they were significantly more abundant in the first location. Climate may

have been a factor in the diminished distribution of these amphipods as water temperature decreased a few degrees in between the time the two collections were taken. However, a sufficient number of specimens were procured in both sites facilitating the on-site filming and observation of behavior.

Two methodologies for the filming of amphipod behavior within a tunicate were developed. Both have advantages and disadvantages which were listed within the results. Overall, the filming within the micro-aquaria appeared to be the most efficient and, if further investigation were done, would be the method selected to do the rest of the filming. However, there were some behaviors that could only be observed while filming in the petri dish so it would be fruitful to use that method depending on the behavior one wishes to observe.

Fourteen observed and interpreted behaviors are listed in Table 2 of the results. These behaviors have been recorded and edited into a panel of behavior clips to be used for future analysis.

The tunicate, which generates a current into the branchial basket and out through the excurrent canal, is an ideal host for amphipods because the constant influx of water is a constant source of nutrition for the amphipod that requires little energy input from the amphipod to acquire. Without this current generated by the tunicate, the amphipod would have to generate a current of its own by rapidly beating its pleopods. This is why pleopod use within the tunicate is more infrequent and the motion is not executed as rapidly as evidenced by the calculated pleopod rates. Furthermore, the amphipods were observed to face into the current in order to maximize feeding regardless of their location with respect to the tunicate.

A comparison of the length and number of pauses in pleopod movement suggests two things. First, that it is probable that the amphipod need not work as hard to generate a feeding current and, second, that the amphipod is acquiring nutrients at a faster rate. The second conclusion can be made due to the fact that the pause in pleopod movement is typically attributed to aggressive cleaning that requires the cessation of pleopod motion.

The observed aggression and non-aggression between amphipods based on kin recognition was a significant indicator of potential eusocial behavior. How *Anamixis cavatura* is able to recognize its kin is unknown. It is possible that there is either an auditory or chemical signal that these organisms exude that helps other organisms determine their relatedness. Gammerids, in fact, possess aesthetascs, club-like chemoreceptors on the flagellar segments on the first antennae, which are believed to function in recognizing either chemical or vibrational stimuli. Thus, further investigation into the physiology of *A. cavatura* may reveal the solution to how they recognize kin.

There appears to be some sort of social structure within the tunicate. Anamorphs appeared to be responsible for the defense of the tunicate and otherwise remained undisturbed in their position of choice within the tunicate. Meanwhile leucomorphs moved almost continuously within and without the tunicate. Leucomorph migration from one tunicate to another may be how colonization of a new zooid occurs.

All the objectives of this experiment were successfully achieved. A system for efficient documentation of amphipod behavior was developed and methods for editing and photographing the acquired video were established. Furthermore, the data collected within this experiment suggests that further investigation into the question of eusociality would be prudent.

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B. List of Figures

1. Figure 1- Tropical, Pacific Amphipod

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2. Figure 2- Ectocommensal Amphipod

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3. Figure 3- Endocommensal Amphipod

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4. Figure 4- Leucothoid Amphipod 1

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5. Figure 5- Leucothoid Amphipod 2

Thomas, J.D., El-Tourky, S.H. Unpublished. Taken December 2009.

6. Figure 6- Anamorph and Leucomorph in Tunicate

Thomas, J.D. and K.N. White. 2009. Dr. Thomas' Amphipod Homepage Leucothoe Taxonomic Database. 1 May 2009.

7. Figure 7- *Anamixis cavatura* on *Ecteinascidia turbinata*.

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8. Figure 8 – Anamorph Edited

Thomas, J.D., El-Tourky, S.H. Unpublished. Taken December 2009.

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IX. Appendix 1

Technology Instruction Sheet

Developed in the Spring and Fall of 2009 for the purpose of the Divisional Honors Thesis

A. VIDEO CAPTURE (from camera)

- Set up the camera in conjunction with the microscope and ensure that there is a mini DV tape in the camera
- Connect the external hard drive to the computer.
- Connect camera to laptop via fire wire 1394 port. (Make sure the ends are plugged in fully!)
 - Turn on camera, restart computer, plug in fire wire cord to laptop and to camera once the computer has restarted.
- Open pinnacle 12
- Once Pinnacle had loaded, click on the CAPTURE tab at the top of the screen.
- Make sure that, in the lower right had corner, the DV full quality capture light is illuminated.
- Click on the folder button next to the word drive on the bottom right hand side of the computer and select the hard drive folder as the folder you will be recording to. Then ensure there is sufficient space on the hard drive to record and edit the length of video you desire.
- Click on the settings button in the lower right hand corner and make sure that the capture format is DV
- Turn on the video camera. Press the red record button.
- Make sure TV standard is NTSC.
- Make sure scene detection is automatic.
- When you want to begin capturing video, press the start capture button.
- When you are done recording press the stop capture button.
- Turn off the Camera. Disconnect the firewire cord.
- Save your capture.

B. VIDEO CAPTURE (from mini DV Player)

- Connect the external hard drive to the computer.
- Turn on the Mini DV player and insert the tape you want to view and edit. Rewind it to the point that you want to begin capturing.
- Connect Mini DV player to laptop via fire wire 1394 port. (Make sure the ends are plugged in fully!)
- Open pinnacle 12
- Once Pinnacle had loaded, click on the CAPTURE tab at the top of the screen.
- Make sure that, in the lower right had corner, the DV full quality capture light is illuminated.

- Click on the folder button next to the word drive on the bottom right hand side of the computer and select the hard drive folder as the folder you will be recording to. Then ensure there is sufficient space on the hard drive to record and edit the length of video you desire.
- Click on the settings button in the lower right hand corner and make sure that the capture format is DV
- Make sure TV standard is NTSC.
- Make sure scene detection is automatic.
- When you want to begin capturing video, press the play button on the mini DV player and press the start capture button in pinnacle.
- When you are done recording press the stop capture button in pinnacle and then stop button on the DV player.
- Eject the tape, Turn off the DV player. Disconnect the firewire cord.
- Save your capture.

C. VIDEO EDITING (Basics)

- Click the edit tab at the top of the screen.
- If the scenes you just filmed don't automatically appear or a different project appears, go to file and click New Project.
- Then click on the folder button to the right of the drop-box at the top of the screen and search for the file you wish to edit.
- Once the scenes appear,
 - Click and drag the scenes you want in you movie to the movie reel area on the lower half of the screen.
 - If you only want a portion of that scene, double click on it and use the screen that pops up to trim the scene to the parts you want.
 - Edit each scene individually by double clicking on it and going through each menu.
 - To alter timing, go to view and click on timeline.
 - To add a title page, click between 2 scenes and click full page title
 - To add a title to the scene, double-click on the scene and click on the second button in the upper-right hand corner twice.
 - Any editing question or confusion, refer to the studio 12 manual downloaded on Sharein's laptop.

D. VIDEO OUTPUT

- When editing has been completed, click on the make movie tab.
- On the right hand side there will be several tabs, click AVI.
- Preview the video, if you like, at this time.
- Press the green button that says "create an AVI file."
- Save the file to a location of your choosing.

E. DVD BURN

- Insert a blank DVD into the computer
- Open Sonic DVD Burner
- Press the “Get Movies” button on the right of the screen that pops up.
- Find the file that you want to burn.
- You can add more files by repeating the procedure mentioned in step 2 and 3.
- To delete a file, click on it and press the delete button on your keyboard.
- You may add or edit the title of your menu if you wish by clicking on the current title. You may also edit the background by selecting the Edit Style button above the title of the video.
- You may preview you files by pressing the play button after a file has been selected.
- When you are ready to burn the DVD, click on the red burn button in the lower, right-hand corner of the Sonic screen and wait for the burning process to complete.
- DVD will pop out when it is finished
- Label and store.

F. CAMERA TO MONITOR

- To attach the monitor to the camera in order to view what was recorded on the camera tape OR to view what is being filmed while also viewing on the laptop, attach the camera to the monitor via the S-video cord.
- Monitor should be on video 2 when connected to the camera via the s-video cord

G. COMPUTER TO MONITOR

- Attach laptop to monitor via blue VGA cord
- Put monitor on PC Mode via input button on top of monitor
- Press Fn F8 (CRT/LCD) button in order to get image on monitor. Use these two buttons to alternate between seeing the image on the computer screen, on the monitor screen and on both screens.

H. VCR to MONITOR

- VCR Cable and Antenna- You can use either an S video cord or you can use a video cable (yellow).
- Yellow end goes into video,
- White end going into Audio L in the VCR,
- L-Mono in the TV and
- the Red end goes into Audio-R in both.

I. Mini-DV Player to Monitor

- Use S-video cord
- Connect first the Mini-Dv player and then the monitor
- Turn on monitor
- Turn on Mini-Dv player
- Make sure monitor is in VCR Mode
- Press play on Mini-Dv Player
- For live feed there will be a slight delay