Eugregarines protists in *Salpa maxima* from the Bahamian Slope

Maya Wolf and Wyatt Miller

Introduction:

All members of the protozoan phylum Apicomplexa (formerly Sporozoa) are parasitic on a range of vertebrate and invertebrate hosts and can be important disease agents (e.g. malarial parasites). Within the Apicomplexa, the Order Eugregarinorida is diverse, supporting 1656 species parasitic on invertebrate hosts, primarily arthropods, but also annelids, spiunculids, hemichordate, and tunicates (Perkins et. al. 2000). This group is unique from other orders of the Apicomplexa because the Eugregarine lifecycle has only two phases of division by multiple fission (i.e. schizogony), sporogony and gametogony. Other orders have all three types of schizogony (sporogony, merogony, and gametogony) in their lifecycle. Within the Eugregarines, members of the genus *Thalicola* infect salps (Perkins et al. 2000).

Five species of *Thalicola* are known to infect six salp species worldwide (Frenzel 1885; Ormieres 1965; Theodorides and Desportes 1975; Perkins et al. 2000). The morphology of all five *Thalicola* species (*T. flava, T. salpae, T. ensiformis, T. fusiformis,* and *T. filiformis*) have been described (Frenzel 1885; Oremieres 1965; Theodorides and Desportes 1975). Theodorides and Desportes (1975) also give prevalence data on four *Thalicola* species found in six known host salp species. Additionally, many if not all, stages in the lifecycle of *T. salpae* and *T. flava* have been noted or described and perhaps the entire cycle of *T. salpae* (Ormieres 1965; Theodorides and Desportes 1968, cited in text only in Theodroides and Desportes 1975). Salps are infected by a sporozoite stage that becomes a trophozoite once successful. Once mature, a single trophozoite often unites with another individual. During this process, called syzygy, one individual attaches its anterior end to the posterior "port" of its "mate," forming two gamonts. In the gamont stage, the posterior individual is considered the male (i.e. satellite) and the anterior the female (i.e. primate). Post-syzygy, both trophozoites begin to shrink, remain attached, and become encysted together. These encysted trophozoites, via fission, produce gametes that fuse and form zygotes that develop into sporoblasts. Each sporoblast will become a spore that will release sporozoites to infect new hosts (Ormieres 1965; Braithwait 197?; Perkins et al. 2000).

While the morphology and developmental stages of *Thalicola* species are well described in salps found in coastal European waters, no studies (to my knowledge) have examined the prevalence of a *Thalicola* species in the same salps found in tropical, albeit deep, waters. Additionally, I have not found literature addressing the ecology of the gregarine/salp host association or any indication of the impact this protist has on its host. Twenty *Salpa maxima* (thus identified based on size, potential distribution, and number of muscular bands in the tunic) from a larger chain were collected off the Bahamian slope using a manned submersible. A *Thalicola* species was found within the intestine of the salps. This gave us an opportunity to address salp host/ gregarine parasite association questions. What is the prevalence and intensity of infection of *Thalicola* sp. in *S. maxima* found along the Bahamian slope? Do these coincide with prevalences reported by Theodroides and Desportes (1975)? What stages are found within a single host and is

there evidence for developmental synchrony or a single infection event? Is there a correlation between host size and the number or size of infecting gregarines? This study addresses these questions.

Methods:

A 20-ft salp chain was collected off Egg Island (25° 30'N, 76° 54'W) in the Bahamas on May 18th, 2008 from a depth of 2134 ft. The manipulator arm of a Johnson-Sea-Link submersible was used to collect the salps and deposit them in an acrylic Bio-box before transporting the samples to the surface. Once on deck, the animals were transferred into a container with chilled saltwater and incubated in a cold room at 12°C.

On the R/V Seward Johnson, the volume of each salp was measured via water displacement. The height and width of each salp was measured using calipers. The tunic of each salp was cut away and the gut nucleus was dissected under an Olympus dissecting microscope. The position of each parasite was noted before removing them to chilled seawater. The length of each gregarine was measured using an ocular micrometer and the stage in the lifecycle noted. Female and male gamonts were measured individually. All stages were documented with a Canon Powershot S3 1S digital camera and the stage micrometers photographed for scale. All gregarines were anesthetized in 7.5% MgCl for 10-15 minutes before preservation. Gregarines were preserved in 100% ethanol for potential genetic analysis and 10% formalin. Additionally, some gregarines were preserved for scanning electron microscopy in 2.5% gluteraldehyde followed by and stored in phosphate buffer wash for shipping. At the Oregon Institute of Marine Biology, we continued preservation in 2% OsO_4 with phosphate buffer washes, followed by an ethanol dehydration series, critical point drying, and sputter coating. These samples were viewed on a Tescan Vega II scanning electron microscope. Gregarine samples were also preserved for transmission electron microscopy but have yet to be viewed.

All statistical analyses were run using SPSS 12.0.1. To test for a relationship between host salp size and the number or size of gregarine parasites, we ran simple or multiple correlation analyses. To test for differences in parasite size according to lifecycle stage we ran a single-factor ANCOVA, with stage as a fixed factor and host salp length as a covariate.

Results:

One hundred percent of the *Salpae maxima* examined (n=17) were infected with a *Thalicola* sp. The morphology and size of this *Thalicola* species eliminates three of the five known *Thalicola* species as possible conspecifics. *Thalicola* sp. shares many characteristics (described below) with *T. salpae.* This *Thalicola* species, like *T. salpa,* has cylindrical and elongate trophozoites that are around 1mm long by 125μm wide (figure 1A). The trophozoites are septate, meaning the two body regions, the protomerite and the deuteromerite, are divided by a septum. Trophozoites have protomerites with longitudinal striations along the pellicule (figure 1A). During head-to-tail syzygy, primite (female) and satellite (male) individuals have different morphologies (figure 1B).

The primite gamont stage has a hemispherical protomerite with striations that end at a simple, button-like epimerite, presumably used for attachment to the host intestine (figure 1F). The male's anterior portion of its protomerite/epimerite is modified into a circular array of fibers, presumably used for attachment to a small invagination in the posterior of the deuteromerite of the female (figure 1G). In contrast to Ormieres (1965) description of *T. salpae*, our species has no prominent fibers in a hemisphere arrangement at the protomerite/epimerite area. Additionally, an interesting note in Lobbe's (1899, cited in Ormieres 1965) list of diagnostic features of *T. salpae* is bright yellow endoplasm, while Frenzel describes their endoplasm as white or blue. Congener *T. flava* has yellow-orange endoplasm (Ormieres 1965). The endoplasm of the *Thalicola* species in our study is red/pink or pink/translucent (figure 1E). This may just be color variation based on the gut contents of the host, though this has not been addressed. For these reasons, assuming our identification of the host is correct, we thus assume this gregarine species to be *Thalicola salpae*, as described by Frenzel (1885), Labbe (1899, in Ormieres 1965) and Ormieres (1965) until an expert can identify it definitively.

The number of *T. salpae* found within the gut or attached to the intestine epithelium of *S. maxima* ranged from two to 30 with an average of 15.59 ± 2.09. We found four stages of the life cycle of *Thalicola* within their salp hosts: trophozoites, gamonts, encysted trophozoites, and gametocysts (see figure 1). Many, though not all, *S. maxima* contained multiple or all life history stages of the protists. Both single trophozoite and gamont stages could be found attached to the intestine and intestinal epithelium of *S. maxima* (figure 1E). Encysted trophozoites and gametocysts were found under the intestinal epithelium of the host but were often buoyant when dissected out of the salp. The average length of trophozoite, gamont (female), gamont (male), encysted trophozoites, and gametocysts are shown in figure 2. There was a significant difference in the length of individuals due to stage, with trophozoites being significantly longer and gametocysts significantly smaller than all other stages ($F_{212,5}= 21.130$, $p<0.001$) (figure 2). Male and female trophozoites joined in syzygy were similarly sized with the female gamont slightly shorter than the male.

There was no significant correlation between host salp length or volume with the number of *T. salpae* individuals (all stages combined) ($r = 0.463$, $p = 0.071$, $n = 16$ and $r = 0.231$, $p = 0.41$, $n = 15$, respectively) (figure 3). However, multiple correlation analyses of salp length versus the number of individuals of each stage suggests that larger salps may host more gamont stages ($r = 0.562$, $p = 0.024$, $n = 16$) (figure 4). Additionally, there was a significant positive correlation between salp length and the length of both the female and male parts of the gamont stage ($r = 0.609$, $p = 0.021$, $n = 14$ and $r = 0.571$, $p = 0.033$, $n = 14$, respectively) (figure 5). There was no significant correlation with salp size and the number or average length of trophozoite, encysted trophozoite, or gametocyst stages.

Discussion:

Our finding of 100% prevalence of *T. salpae* supports the findings of Theodroides and Desportes (1975) in the Alpes-Maritimes area. However, *T. salpae* do not have 100% prevalence on all its host species. *Thalicola salpae* parasitize two other hosts,

Salpa fusiformis and *Ihlea punctata,* at prevalences of 16% and 82%, respectively (Theodroides and Desportes 1975). Finding an average of 15 gregarines per host is not surprising as Frenzel (1885) and Ormieres (1965) mention "numerous" *T. salpae* within each host. I have not found quantitative intensity data on this genus yet, however.

Multiple stages, often all four, were found within a single host, suggesting that the host is infected periodically, not by a single mass infection of sporozoites. If 100% of the individuals in a colony of salps are infected by *T. salpae* that release sporozoite infective stages consistently over time, this may lead to frequent horizontal transmission to neighboring salps. If, however, sporozoites are buoyant, a possibility considering the buoyancy of encysted trophozoites and gametocysts, these infective stages may have greater dispersal potential, even if encountering another salp host is unlikely. One would expect the lengths of the four stages to differ as trophozoites and gamonts are elongate and encysted trophozoites and gametocysts elliptical and spherical, respectively. However, I did not expect trophozoites to be significantly longer than male and female gamonts as the latter are just attached individuals of the former. This suggests that the fusion of two trophozoites may involve shortening of one or both individuals. The invagination at the posterior end of the deuteromerite of the primite gamont and the modification of the protomerite of the satellite gamont to fit this invagination may make both gamonts slightly "shorter" (Ormieres 1965) Additionally, we expected cysts to be only slightly smaller than encysted trophozoites as they contain, presumably, the same volume of protist material. This may just be a result of measuring the long axis of an ellipse versus the diameter of a sphere.

We expected to see a significant correlation between the size of the salp and the number of *T. salpae* within the host. Many studies have found large hosts more often parasitized and in greater intensity than small individuals (Rothschild 1936, 1941; Baudoin 1974; Sousa 1983). This is probably because larger and older individuals have a larger body volume to hold parasites and have been susceptible to infection longer than small individuals (Baudoin 1975; Sousa 1983). In contrast, our data suggest there is no correlation between the size of the host (for all gregarine stages combined) and the number of *T. salpae*. This could be because the parasites are not space or energy limited even at their densest (i.e. 30 individuals) in smaller salps. If there were an average of 15 *T. salpae* per host, with most under a millimeter, their occupied space may not be enough relative to the host to cause competition among gregarines. Alternatively, I do not know the duration of the gregarine lifecycle nor have I found it in the literature; the turnover rate of individuals (spores and sporozoites existing the host versus sporozoites infecting) may be a delicate balance that maintains an optimal number of parasites within the salp. Additionally, there may not have been enough variation in salp size to see a difference. We also should have measure the tight nucleus of the gut instead of the whole tunic as the area of the intestine appears to be the only are of occupation for *T. salpae*. Also, encysted trophozoites and gametocytes not attached to the gut may not be occupying valuable attachment space. The significant correlation between salp size and number and length of male and female gamonts suggests that host size may only be important during certain stages. Gamont stages were found attached to the intestine and gut epithelium suggesting they are actively gaining nutrition from the host at this stage and occupying significant space within the host. Larger hosts may be able to better accommodate more gamonts that may grow larger if they remain "feeding" within the host for a longer

duration. However, I would image the statistically larger trophozoites would also show this significant positive correlation if it is ecologically important as they also are attached to the intestine and intestinal lining at this stage.

From these data, little can be deciphered about the impact of *T. salpae* on the its host. It may be that the small area occupied by the gregarine parasites relative to its hosts inflicts negligible negative effects. This is particularly supported by the 100% prevalence of this parasite on its hosts. If the parasites were particularly virulent they would quickly eliminate the entire host chain and thus, themselves.

Conclusion:

We are uncertain of the precise identification of the salp and its gregarine parasite but are tentatively calling them *Salpa maxima* and *Thalicola salpae*, respectively, based on morphological characters, size, and known associations. 100% of S. maxima were infected with an average of 15 *T. salpae*. Four stages of the lifecycle of *T. salpae* were found in the hosts: trophozoites, gamonts, encysted trophozoites, and gametocysts. There was no significant correlation between host length and the number of parasites when stages were combined. However, there was a significant positive correlation between host size and the number and length of male and female gamonts, suggesting this stage may utilize or grow more efficiently in larger salps.

Literature Cited

Baudoin, M. (1975). Host castration as a parastic strategy. *Evol.* **29**:335-352.

Braithwaitte, L. (?). Invertebrate Zoology: Lab Manual. P. ?

Desportes, I. and J. Theodorides. (1969). Ultrastructure de la Gregarine *Callyntrochlamys phronimae* Frenzel; etude compare de son noyau avec delui de *Thalicola salpae* (Frenzel) (*Eugregarina*). *J. Protoz.* **16**: 449-460.

Frenzel, J. (1885). Uber einige in Seethieren lebende Gregarinen. *Arch. F. Mikr. Anat.* **24**: 545-588.

Oremieres, R. (1965). Recherches sur les Sporozoaires parasites des Tuniciers. *Vie et Milieu* **15**: 823-946.

Perkins, F.O., J. R. Barta, R.E. Clopton, M.A. Peirce, and W. J. Upton. (2000). Phylum Apicomplexa in The Illustrated Guide to the Protozoa. 2nd Edition. Eds. Lee, J.J., G.F. Leedale, and P. Bradbury. Allen Press. Lawrence, KS p.689.

Rothschild. (1936). Gigantism and variation in *Peringia ulvaoe* Pennant 1777, caused by infection with larval trematodes. *J. Mar. Biol. Ass. U.K.* **20**:537-546.

--(1941). The effect of trmatode parasites on the growth of Littorina neritoides. *J. Mar. Biol. Ass. U.K.* **25**:69-80.

Sousa, W.P. 1983. Host life history and the effect of parasitic castration on growth: a field study of *Cerithidea californica* Haldeman (Gastropoda: Prosobranchia) and its trematode parasites. *J. Exp. Mar. Biol. Ecol.* **73**: 273-296.

Theodorides, J. and I. Desportes. (1975). Sporozoaires d'invertebres pelagiques de Villefranche-sur-mer (etude descriptive et faunistique). *Protistologica* **2**: 205-220.

Wrobel, D. and C. Mills. (1998). Pacific Coast Pelagic Invertebrates. Monterey Bay Aquarium. p. 108.

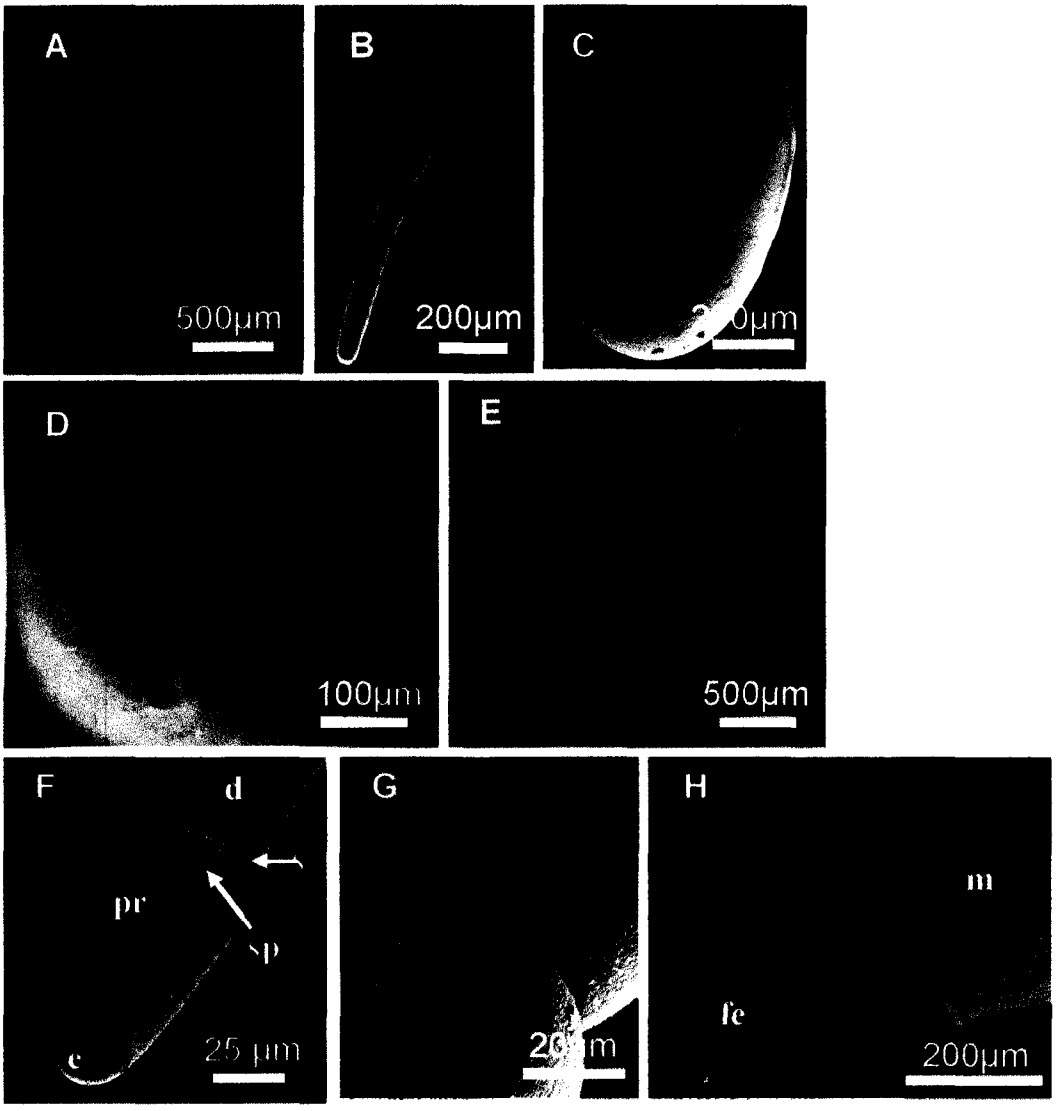


Figure 1: *Thalicola salpae* **A.** Trophozoite **B.** Two gamonts (female anterior, male posterior) in syzygy. **C.** Encysted trophozoite **D.** Gametocyst **E.** Gamont stages attached to the host intestinal epithelium **F.** Striated pellicle and protomerite, then button-like epimerite. **G.** Junction of female and male gamonts **H.** Male gamont anterior and posterior female gamont. d=deuteromerite, e=epimerite, fe=female (or primite), m=male (or satellite), pr=protomerite, s=septum, sp=striated pellicle

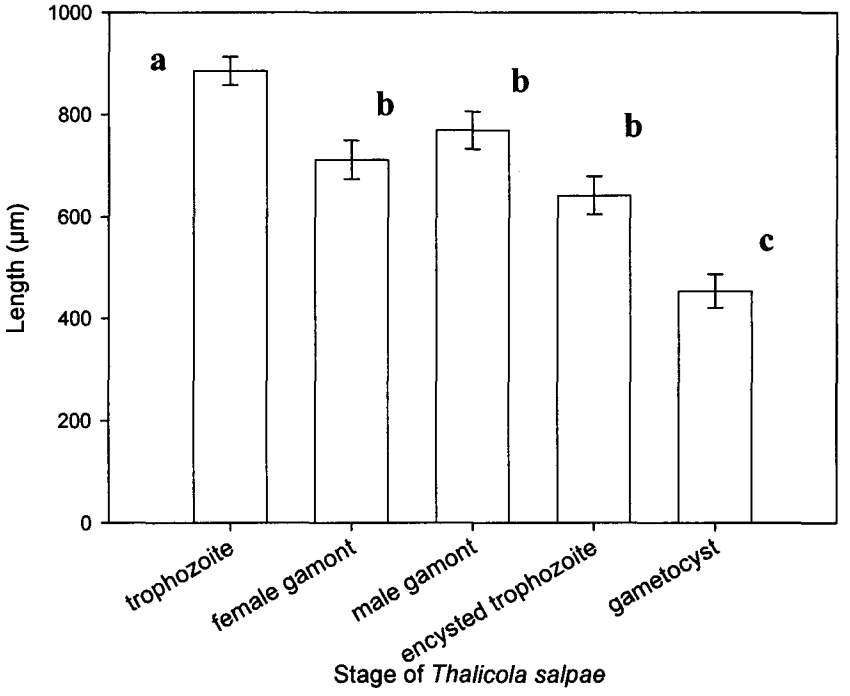
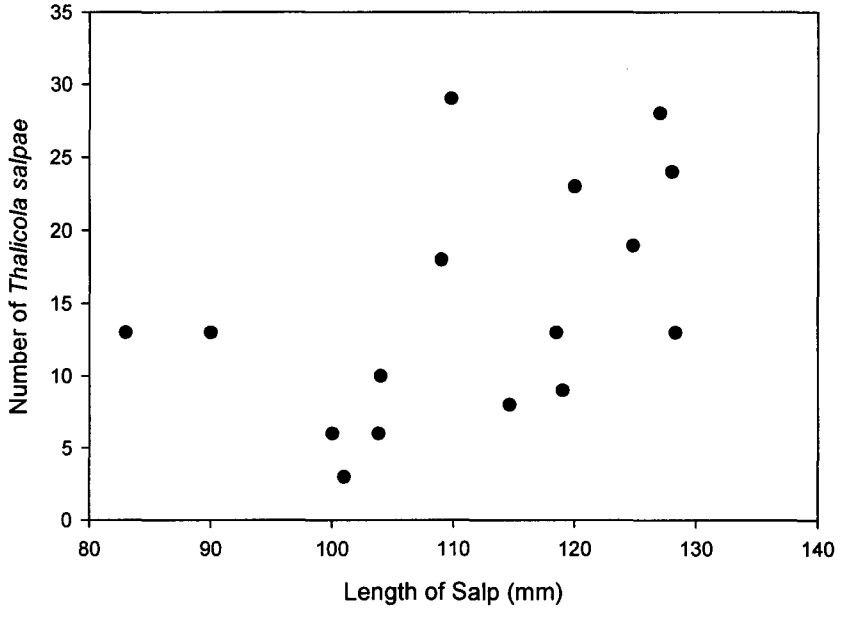


Figure 2: Lengths of different stages in the life cycle of *Thalicola* sp. Letters designate significant differences. ($F_{212,5}$= 21.130, p<0.001).



3,

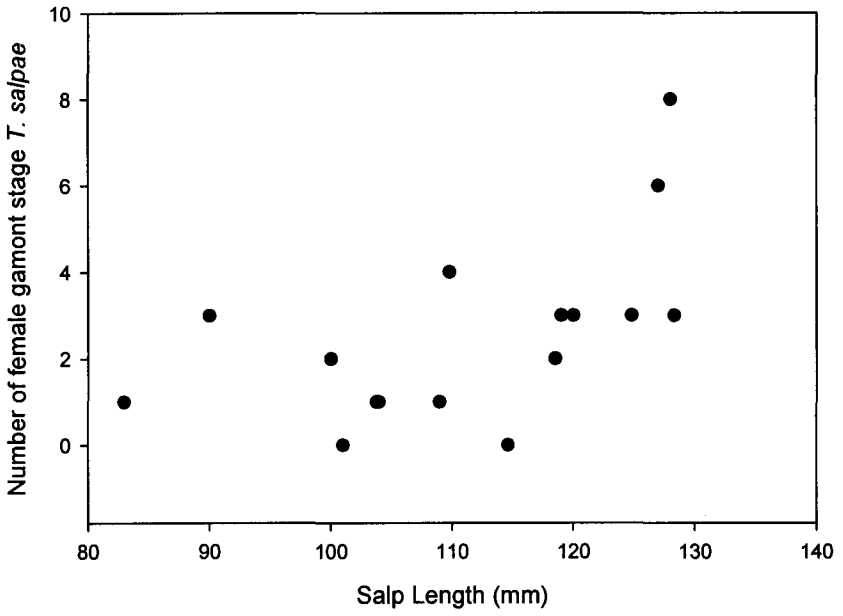


Figure 4: Salp Length vs. Number of female gamont stages of *Thalicola salpae* (r=0.562, p=0.024, n=16).

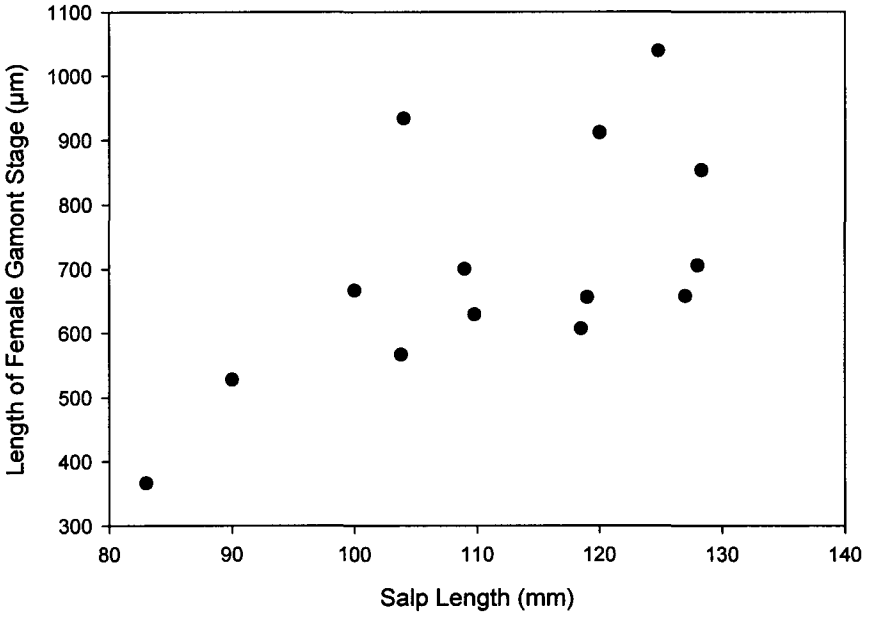


Figure 5: Salp length vs. female gamont length (r=0.609, p=0.021, n=16).