

Crawling with Worms: A look at two symbiotic relationships between polychaetes and urchins from the Bahamas
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Introduction

A central focus in understanding the deep-sea ecosystem is the relationship between biotic factors. As with most habitats the majority of deep-sea interactions are direct, species compete for food, reproduction, predator/prey, etc. However, there are some organisms that have formed an even closer association. This symbiotic relationship between species can be mutual, commensal, or parasitic. Marine polychaetes form some of the most unusual and interesting mutual, commensal, and parasitic relationships in the animal kingdom. This study looked at just two of the 713 documented polychaete symbiotic interactions (Martin and Britayev, 1998).

Archeopneustes hystrix has a species-specific commensal or parasite, *Benthoscolex cubanus*, spending the majority on its life feeding in the gut (Emson et al. 1993). What are the possible effects of this endosymbiont on the host? *A. hystrix* and *B. cubanus* were collected and studied in order to try and unravel this relationship. This study led to the analysis of two other Caribbean urchins, *Conolampus sigsbeii* and *Paleopneustes cristatus*, for signs of polychaetes.

Ectosymbionts are organisms that live on the exterior of the host (Rohde, 2005). Although the majority of ectosymbionts have no pathological effect on the host, there are some instances where an ectoparasite infestation has increased stress on the host. *Neoheterobothrium affine*, a marine gill fluke, has been associated with mortalities of olive flounder in Japan since the 1990s (Rohde, 2005). The salmon louse, class Copepoda, has been documented to cause haemorrhages in the skin to complete osmoregulatory failure and death in Atlantic salmon (Tully and Nolan, 2002). When a hesperionid worm was found with the deep-sea Caribbean urchin *Linopneustes longispinus* it was important to look into the possibility of another ectoparasite causing pathological effects on its host.

Materials and Methods

On May 14 through May 23, 2008 sea urchins were collected from the mud and sand floors off the shores of the Bahaman islands. The sites were mud mounds or gentle slopes around Paradise Island, Morgan's Bluff, and Stanard Rock. A Johnson-Sea-Link submersible was used to collect *Conolampus*, *Archeopneustes*, *Linopneustes*, and *Paleopneustes* between 1590 ft to 2200 ft below sea level. The specimens were collected using an acrylic bucket scoop or suction hose on the manipulator arm of the submersible. The specimens were transported to the surface in collection buckets or a mounted acrylic bio-bin. Once on deck, the urchins were transferred into buckets and containers incubated in a cold room at 12°C. All urchins were housed together except for *Linopneustes* collected during dive 3659 on May 22, 2008 where individuals were housed in separate containers.

Urchins were inspected for possible external worm symbionts then dissected to look for internal symbionts. Before dissection water displacement was used to give an approximation of overall urchin volume. Urchin height, width, and length were measured using calipers. Once dissected gonad volume was measured using water displacement. If a worm was found the length was measured using calipers. All worms were anesthetized in 7.5% MgCl for 10-15 minutes before preservation. Worms were preserved in 100% ethanol for potential genetic analysis or 10% formalin. Additionally, some worms 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% OsO₄ 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. Worm samples were also preserved for transmission electron microscopy but have yet to be viewed. All statistical analyses were run using SPSS 12.0.1.

Results:

Of the four urchin species dissected, *Conolampus sigsbeii* (n=7) and *Paleopneustes cristatus* (n=8) did not have any associated polychaetes, while *Archeopneustes hystrix* (n=16) and *Linopneustes longispinus* (n=24) had endo- and ecto-symbiotic polychaetes, respectively (figure 1).

Archeopneustes hystrix

Archeopneustes hystrix were collected from three different sites, Paradise Island (n=8), Morgan's Bluff (n=4), and Staniard Rock (n=4). *A. hystrix* from Morgan's Bluff were significantly smaller than urchins from Paradise Island and Staniard Rock (figure 2A). There was no significant difference in gonad indices of *A. hystrix* from different sites, but those from Morgan's Bluff had the smallest indices (figure 2B). Prevalences of the fireworm, *Benthoscolex cubanus*, in *A. hystrix* from Paradise Island, Morgan's Bluff, and Staniard Rock were 50%, 100%, and 25%, respectively. While only one *A. hystrix* from just Morgan's Bluff had two *B. cubanus* inside its gut, there were four worms in the container housing these urchins. This strongly suggests double infections, given the 100% prevalence at this site. There is no evidence to suggest multiple infections in *A. hystrix* from Paradise Island or Staniard Rock. Although there was no significant difference in the length of *B. cubanus* found within urchins verses swimming urchin buckets, these "escaped" worms were generally larger than worms found inside the hosts ($F_{8,2}=3.922$, $p=0.095$, $\text{power}=0.385$) (figure 3).

Only Paradise Island urchins had enough "infected" and "uninfected" individuals to assess the potential impact of the worms on growth or reproduction of the hosts. There was no significant difference in the size (volume) or gonad indices of infected and uninfected *A. hystrix* ($F_{8,2}=0.007$, $p=0.934$, $\text{power}=0.051$ and $F_{8,2}=2.79$, $p=0.146$, $\text{power}=0.291$, respectively) (figures 4A and 4B). Additionally, there was no significant correlation between host urchin size and worm length ($r=-0.0359$, $p=0.382$) (figure 5).

Linopneustes longispinus

No polychaete associates were found within the gut or test of *L. longispinus* (n=31). Unidentified polychaetes were found crawling on *L. longispinus* and swimming in containers holding urchins (figure 6 and 7). Preliminary transmitted light and scanning electron microscopy images suggest this polychaete belongs in the family Hespionidae.

The worm has neuro and noto chaete similar to documented species in Hespionidae. It also has 4 palps and central palp which includes posterior palps suggesting hespionid. There isn't a jaw within the pharynx only a chitinous, hard ridge. Some individuals retained their prototroch suggesting the organism to be juvenile (fig 6).

Worms from the first Paradise Island collection (May 19) are not included in the following results as they could not be paired with a specific urchin. A second collection of *L. longispinus* (May 22) isolated each urchin in a separate carousel container with surrounding mud enabling us to pair urchins and their specific worm associates. Often, one worm was found crawling along the outside of the urchin test (fig 7). The prevalence of this ecto-symbiotic hespionid worm was 67%, including worms in the urchin bucket or mud surrounding the urchin. In mud collected under two *L. longispinus*, we found two worms per sample, suggesting multiple worm associates per urchin. There was no significant difference in the size (volume) or gonad indices of *L. longispinus* with and without hespionid associates ($F_{12,2}=1.87$, $p=0.205$, $p=0.232$ and $F_{12,2}=1.44$, $p=0.260$, power=0.190, respectively) (figure 8A and 8B). Additionally, there is no significant correlation between urchin volume and worm length ($r=0.281$, $p=0.542$, $n=7$) (fig 9).

Discussion

The results from the *A. hystrix* suggests that larger worms may be more motile, likely to evacuate and/or move among the host (as suggested in Emson et al. 1993). *B. cubanus* could be sensitive to disturbance. The Morgan's bluff urchins were larger with smaller gonad which could possibly be because of poor diet or newly spawned. There is also a possibility of decreased gonad due to energy loss from double infections. There is no evidence to suggest a significant redirection of host energy/resources from the urchin to the worm. It is therefore believed to be more of a commensal relationship as *B. cubanus* consumes diatoms within the intestine of the urchin. Perhaps there is marginal energy drain but due to the small sample size this relationship cannot be fully determined.

L. longispinus probably has a commensal relationship with the hespionid worm. The worm will be sent for further identification. For further study, it would be important to separate each urchin while collecting. We should take more video and photos of the urchin before collection in order to capture this relationship. We would also suggest collecting mud from the area and looking for the worm to see if it was just coincidence we caught both although this is unlikely.

Literature Cited

- Emson, R.H., Young, C.M. and G.L.J. Paterson 1993. A fire worm with a sheltered life: studies of *Benthoscolex cubanus* Hartman (Amphinomidae), an internal associate of the bathyal sea-urchin *Archeopneustes hystrix* (A. Agassiz, 1880). *Journal of Nat Hist.* 27:1013-1028.
- Martin, D. and T.A. Britayev 1998. Symbiotic Polychaetes: Review of Known Species. *Oceanography and Marine Biology: an Annual Review* 36:217-340.
- Rohde, Klaus. Marine Parasitology. Collingwood, Australia: CABI Publishing, 2005.
- Tully, O. and D.T. Nolan 2002. A review of the population biology and host-parasite interactions of the sea louse *Lepeophtheirus salmonis* (Copepoda:Caligidae). *Parasitology* 124:S165-S182.

Pictures and Figures

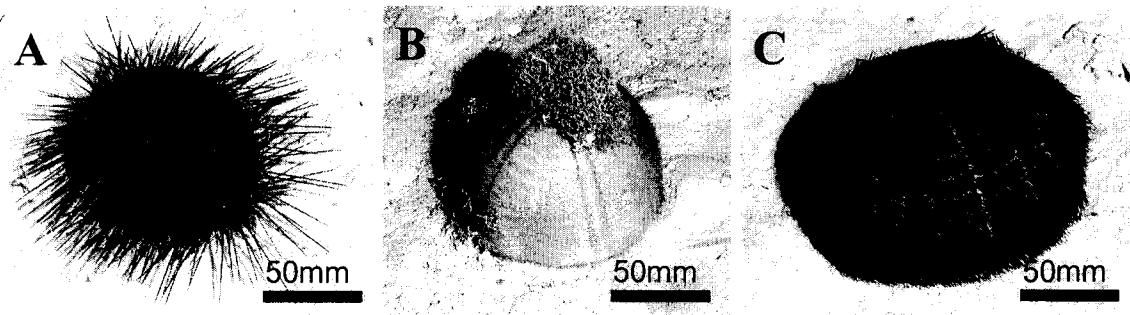


Figure 1: **A.** *Archeopneustes hystrix* **B.** *Conolampus sigsbeii* **C.** *Paleopneustes cristatus*

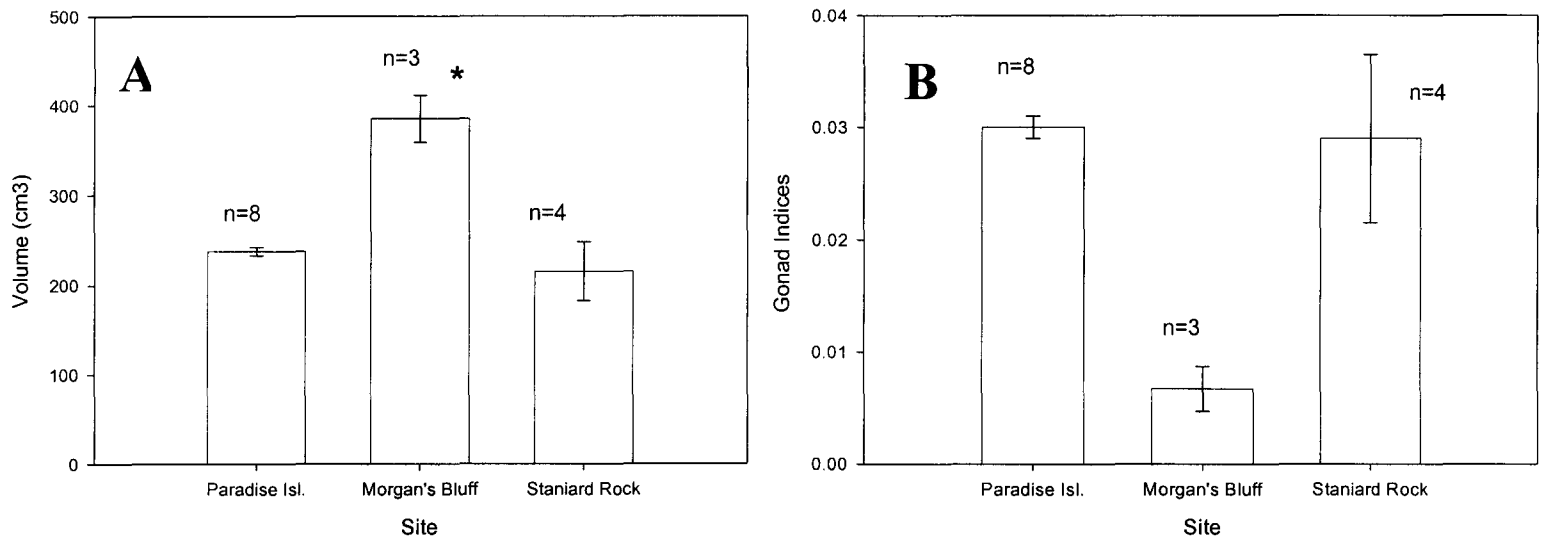


Figure 1: **A.** Volume of *Archeopneustes hystrix* at three sites. Asterick designated a significant difference ($F=4.743$, $p=0.03$) **B.** Gonad indices of *A. hystrix* at three sites.

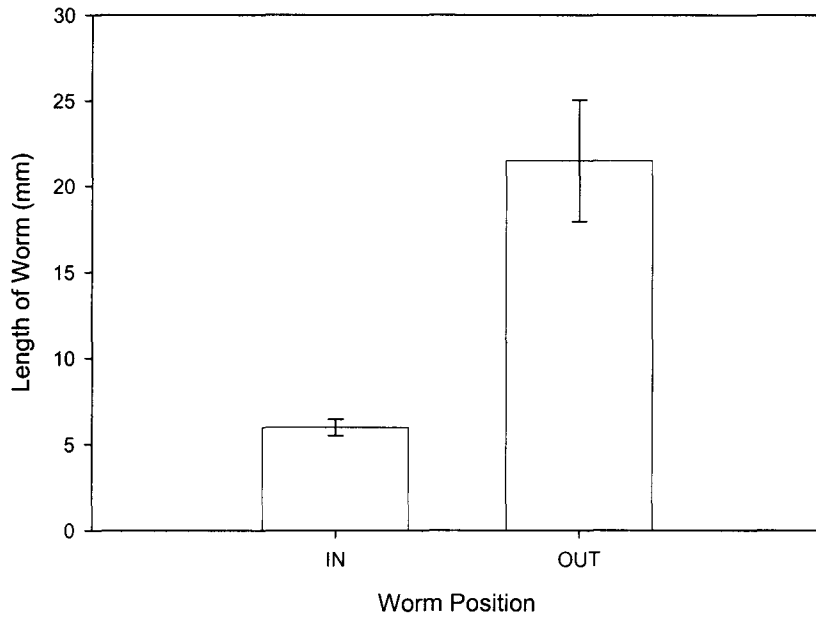


Figure 3: Length of worms found within *Archeopneustes hystrix* and evacuated worms found in containers housing urchins. ($F_{8,2}=3.922$, $p=0.095$, power=0.385)

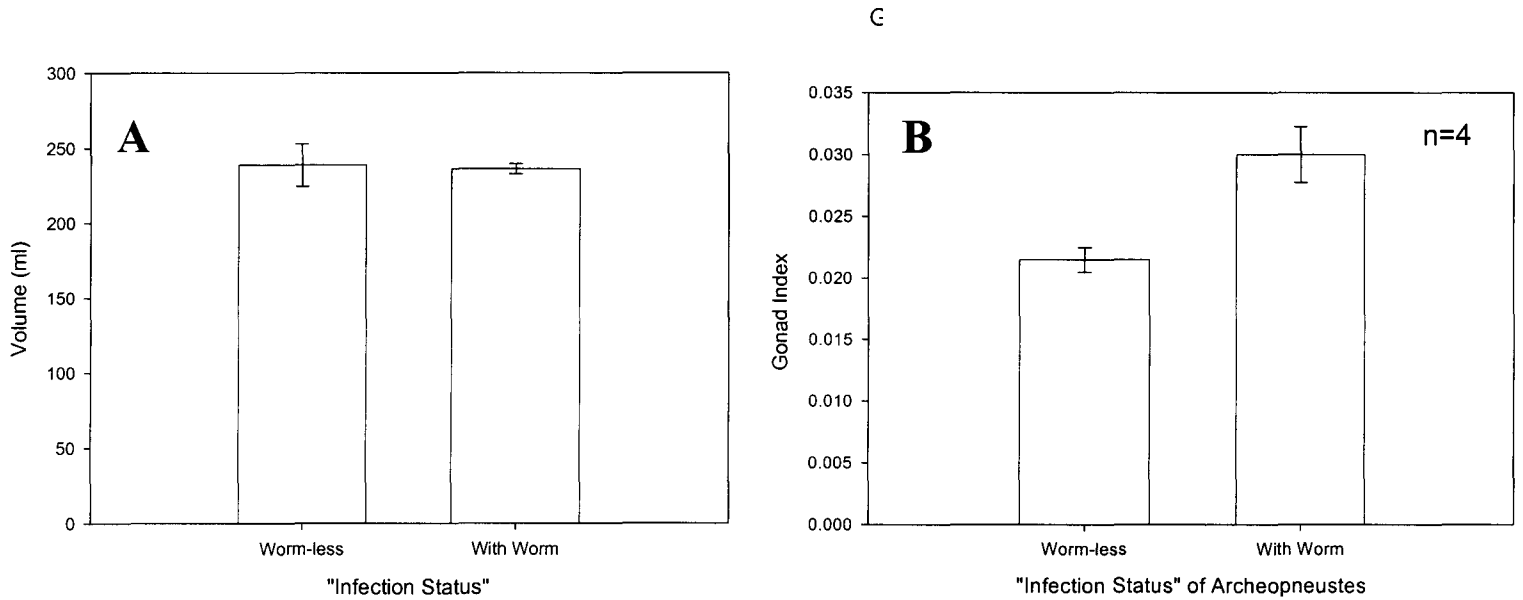


Figure 4: **A.** Volume of *Archeopneustes hystrix* with and without *B. cubanus* ($F=0.007$, $p=0.934$, $n=4$). **B.** Gonad indices of *A. hystrix* with and without associated *B. cubanus* ($F=2.791$, $p=0.146$, $n=4$).

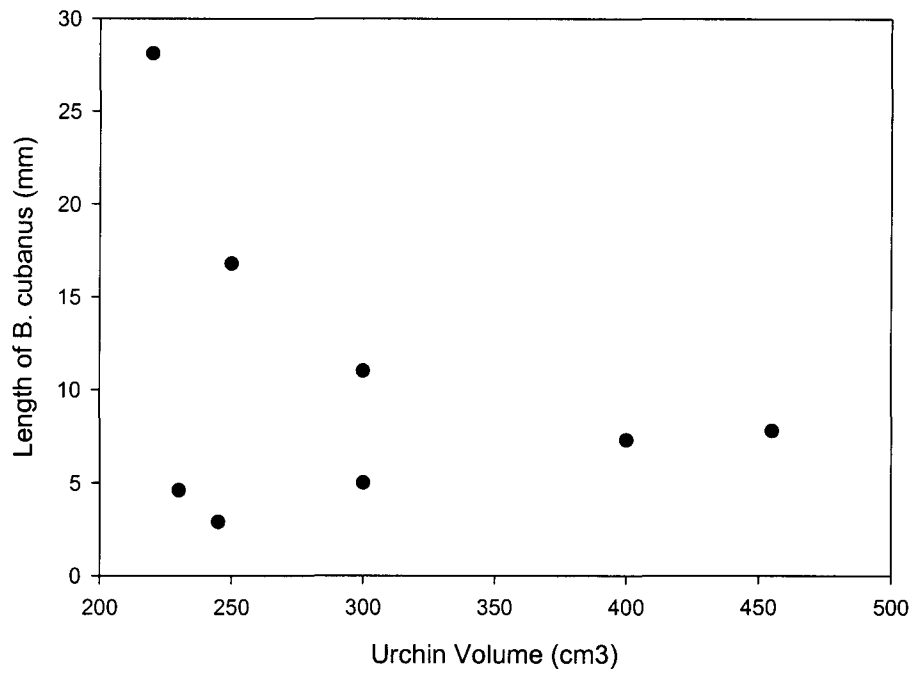


Figure 5: Correlation analysis of volume of *Archeopneustes hystrix* and length of *B. cubanus* ($r = -0.0359$, $p = 0.382$).

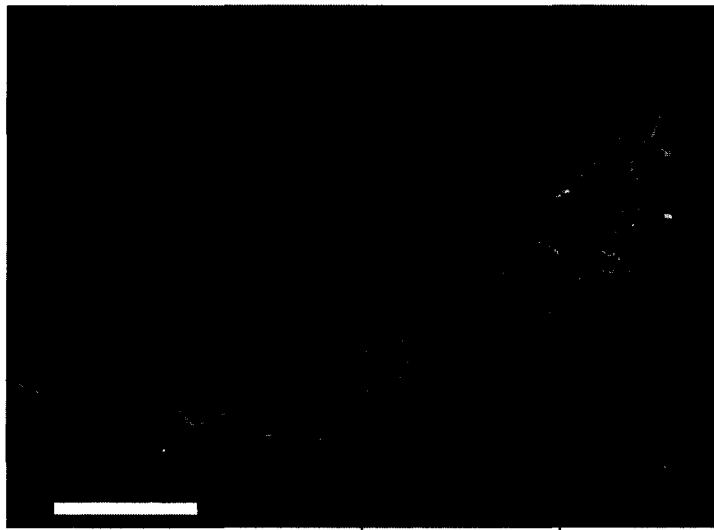


Figure 6: SEM of the ectoassociate hespionid worm of *Linopneustes longispinus*. Scale bar is 1mm.

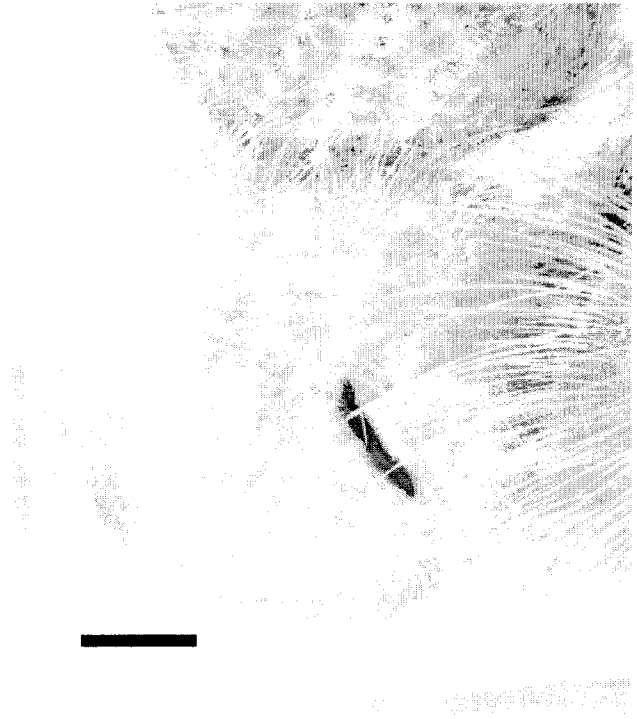


Figure 7: *Linopneustes longispinus* with ectosymbiotic hespionid worm. Scale bar represents 25mm.

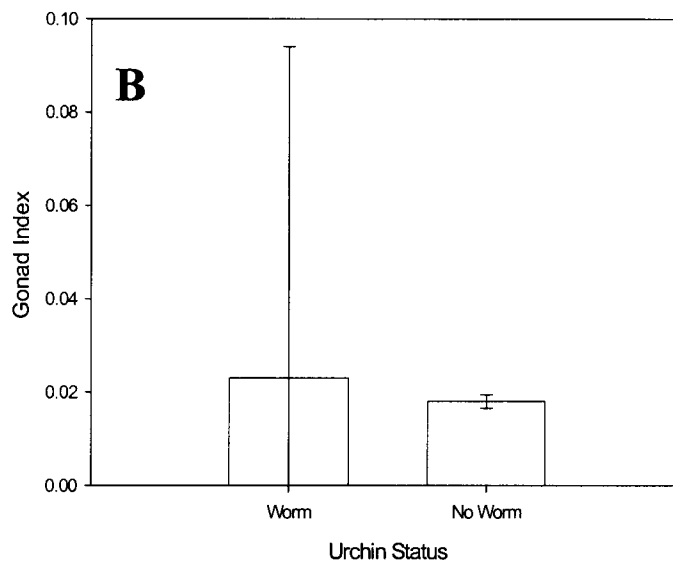
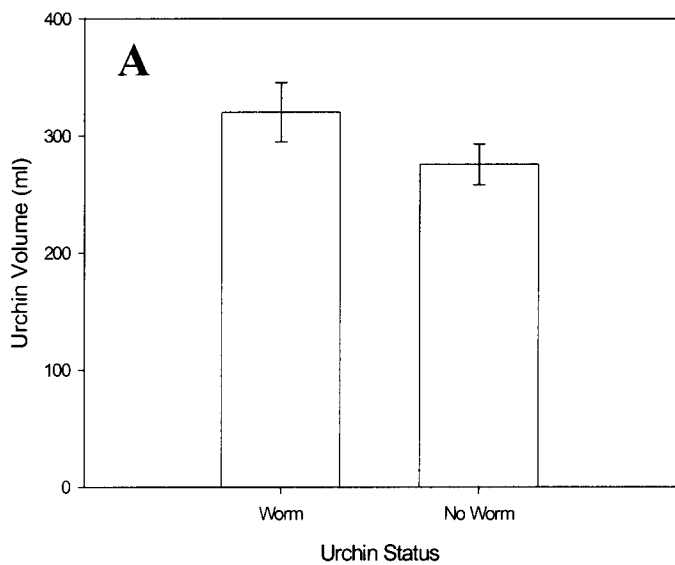


Figure 8: **A.** Volume of *Linopneustes longispinus* with and without the associated hespionid polychaete ($F_{12,2}=1.87$, $p=0.205$, $p=0.232$, $n=$). **B.** Gonad indices of *L. longispinus* with and without the associated hespionid polychaete ($F_{12,2}=1.44$, $p=0.260$, $power=0.190$).

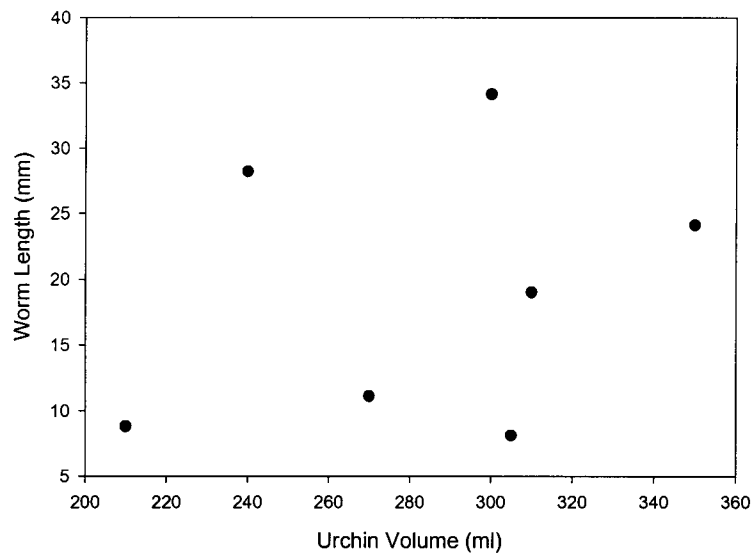


Figure 9: Correlation of *Linopneustes longispinus* volume and hespionid worm length ($r=0.281$, $p=0.542$, $n=7$).