

Co-evolution or easy exploitation? Trematode prevalence, diversity and lifecycles in endemic gastropods of Lake Tanganyika, East Africa

Students: Sarah Collins and Julie Hopper

Mentor: Ellinor Michel

Introduction

Lake Tanganyika's ancient age (12 MY) and remarkably diverse array of endemic flora and fauna are reason to expect co-evolutionary patterns among its inhabitants. Interactions between gastropod hosts and trematode parasites present a potential arms race in the lake that is relatively unstudied despite potential impacts on evolution of endemic species and ecosystem function. In this study, we examine whether differences in parasite prevalence and diversity correlate to depth, site characteristics, host gastropod morphology and reproductive strategies at the population level. We also aimed to determine possible second intermediate and final hosts in the parasite lifecycle.

Elucidation of the trematode lifecycle and identification of subsequent hosts is an important aspect of an examination of patterns in parasite prevalence and diversity. While past studies have noted differing parasite prevalence by depth and site (Muthukrishnan 2004, McIntyre et al 2005), the identity of these parasites remained unknown and could be indicative of future hosts in the trematode lifecycle. In a typical trematode parasite lifecycle, molluscs either consume digenean trematode eggs deposited in their food source or trematodes penetrate the mollusc host in the free-living, ciliated miracidium stage. After infection, trematodes progress to a sporocyst stage, and develop asexually to rediae. From this, embryos hatch into the cercaria stage. Subsequent hosts are infected either by consuming an infected mollusc or by cercariae that have emerged into the water column and penetrate the next host. The cercariae develop either directly to the adult worm stage, or to metacercariae, which are consumed by a third and final host. Once the adult worm has developed, the final host expels the adult trematode's eggs in fecal matter and the cycle continues (Whitfield 1993, pp. 205).

We focused on *Lavigeria nassa* as a gastropod host. *Lavigeria nassa* is commonly found on rocky substrate throughout the littoral zone of Lake Tanganyika. Wide-ranging distribution and population-level genetic variation (Michel et al. 2005) make *L. nassa* an attractive study organism to examine co-evolution and local adaptation. Previous findings show variable parasite prevalence across sites in the Kigoma area of Lake Tanganyika and a positive correlation between parasite prevalence and depth from 1-10 meters (Muthukrishnan 2004, McIntyre et al 2005). For cross-species comparison, we also examined a small number of *Vinundu guillemei* and *Paramelania damoni*.

Parasites can affect the growth, fecundity and survival of their hosts (Sorensen & Minchella 1998). Gastropods in a highly parasitized population may grow more rapidly and reach maturity earlier in order to maximize their reproductive success before castration (Fredensborg & Poulin 2006). Recent work in other study systems note morphological changes in infected gastropods including gigantism and dwarfism, (Miura et al 2006), but previous research on *L. nassa* revealed no marked morphological differences between parasitized and unparasitized individuals (Athuman 2004, Callier 2006). Similarly, a shift in brood size or reproductive age could also indicate host life history changes in response to parasites (Fredensborg & Poulin 2006). For example, gastropods in sites with high parasite prevalence might be predicted to brood fewer young to invest more energy to each embryo, increasing their chances of survival. Behavioral changes could induce predators to consume infected hosts, such as movement toward habitat regions of consumers or tantalization of predators (Miura et al 2006). We aimed to examine some of these common host responses to trematode parasites by recording basic morphometric data and examining the brood size of reproductive *L. nassa* females at sites where we had documented parasite prevalence and diversity.

In addition to causing modifications in the host and its role in the environment, trematode species diversity and prevalence may indicate ecosystem diversity and health at sediment-disturbed sites compared to undisturbed sites (Gardner & Campbell 1992). Anthropogenic increases in sedimentation in some areas of the lake correlate with individual and community level changes in density, species richness, parasitism, and predation in Lake Tanganyika gastropods (McIntyre et al. 2005). Increased sedimentation could prevent the egg or miracidia from contacting the snail host (McIntyre et al. 2005). We sought to determine if increased sediment load would correlate with parasite prevalence and diversity by comparing a relatively undisturbed (anthropogenically) site, Jakobsen's Beach, to a sediment impacted one, Hilltop Point.

Methods

Three collection sites were selected in the Kigoma area of Lake Tanganyika: south of Jakobsen's Beach ("JKB-O"- S 04° 54.944' E 29° 35.807'), between the two beaches at Jakobsen's ("JKB-C"- S 04° 54.635' E 29° 35.905'), and along the South side of TAFIRI Bay on "Hilltop Point" ("HTP"- S 04° 53.227' E 29° 36.795' (Muthukrishnan 2004)). Both Jakobsen's sites are bordered by a protected forest area, and predominantly characterized by large rocky substrate. A small sandy bay and beach separates the two sites at Jakobsen's. The Hilltop area is characterized by increased sedimentation and nutrient availability (McIntyre et al. 2005). The substrate is composed of smaller cobbles, conglomerate, pebbles and stromatolites.

At each site, specimens were collected using SCUBA on three transects perpendicular to the shoreline. Approximately 30 *L. nassa* were collected at 5m, 10m, 15m and 20m along each transect. For cross-species comparison of parasite prevalence and diversity, other species in the study area, *V. guillemei* and *P. damoni*, were collected as abundance permitted. *Paramelania damoni* was found intermittently on soft substrate at Hilltop beach below 10m and *V. guillemei* was found at both Jakobsen's sites below 10m.

Snails were scrubbed prior to processing to remove any algal growth on the shell. Using digital calipers, the height (maximum distance from apex to aperture, parallel to the axis of coiling), width (maximum distance perpendicular to the coiling axis) and lip thickness were recorded for each snail (Wagner 2004). The number of scars due to attempted crab predation visible to the naked eye was also noted. Snails were cracked and examined under a dissecting microscope for trematode infection. The broods of all reproductive females were preserved in 95% ethanol for further analysis. Trematodes were categorized by morphotype and specimens were preserved in 10% formalin for approximately 24 hours and thereafter in 70% ethanol for further identification.

All statistical analyses were performed using JMP IN, SAS Institute. Trematode prevalence was calculated as the number of hosts infected by one or more individuals of a parasite species divided by the number of hosts examined (Bush et al. 1997).

Results

Parasite diversity and prevalence:

Lavigeria nassa had significantly different parasite prevalence between the different sites and depths (Figure 1; likelihood ratio test: site: $\chi^2=9.363$, $df=2$, $p=0.0093$; depth: $\chi^2=12.116$, $df=3$, $p=0.0070$), but parasite prevalence among transects at each site did not differ significantly (likelihood ratio test: $\chi^2=4.198$, $df=2$, $p=0.1226$). Hilltop had significantly lower parasite prevalence than found at either of the two Jakobsen's sites (Figure 1; likelihood ratio test: $\chi^2=4.8885$, $df=2$, $p < 0.05$). Parasite prevalence at all sites was significantly lower at 5 meters depth than at 10, 15, or 20 meters (Figure 1, likelihood ratio test: $\chi^2=6.6840$). Diversity of trematodes was significantly different among sites (Figure 2; likelihood ratio test: $\chi^2=16.260$, $df=8$, $p=0.0388$), however 20% of the cells had values less than 5, giving an error reading for the analysis. Diversity of trematodes among depths did not differ significantly (likelihood ratio test: $\chi^2=6.201$, $df=12$, $p=0.9056$), but trematode morphotypes 'E' and 'F' were more prevalent at certain depths (Figure 3).

Additional gastropod host infections

Of 106 total *P. damoni* collected, 16 were infected by trematode parasites. In our preliminary identification, the 75% of 16 parasitized hosts were infected with morphotype 'C': a cyathocotylid; 18.8% were infected with 'G': Pleurolophocercous cercariae; and 16.3% were infected with 'H': Virgulate or Armatae cercariae. There was no significant difference in the number of parasitized *P. damoni* and *L. nassa* at Hilltop (Likelihood ratio: $\chi^2=3.232$, $df=1$, $p<0.0722$). Out of the 28 *V. guillemei* dissected, one host was parasitized with morphotype 'E': a sanguinicolid trematode.

Trematode identification (all occurred in *L. nassa* with the exception of morphotype C):

We identified five basic trematode morphotypes in this study:

- Lophocercous-apharyngeate cercariae, belonging to the family Sanguinicolidae, which directly encysts from the gastropod first intermediate host to a definitive fish host. (Figure 4).
- Morphotype E, remains unidentified but may be related to the Echinostomidae (Figure 5).
- Pleurolophocercous cercariae, belonging to the families Cryptogonimidae, Opisthorchidae or Heterophyidae, all of which encyst in a definitive fish host following gastropod infection (Figure 6).

- Virgulate or Armatae cercariae (Figure 7).
- Morphotypes B and C. Morphotypes B and C were found in *L. nassa* and *P. damoni* respectively. Thought to belong to the family Cyathocotylidae, they are found in the mantle cavity and gut lumen of the host snail rather than the gonads (Figures 8 and 9).

Scarring, morphometrics and parasite prevalence

Scarring frequency due to attempted crab predation differed significantly among sites, with the highest frequency noted at Hilltop Point (Figure 10), (ANOVA: F ratio= 8.9642, p=0.0001; Tukey-Kramer HSD: p< 0.05) and was borderline significant with depth (ANOVA: F-ratio: 2.5990, p=0.0509). Both height and sex varied significantly with scarring frequency and a significant interaction existed between height, sex and scarring frequency (ANOVA: height: F=39.7743, df=1, p <0.0001; sex: F=8.6333, df=2, p<0.0002; height*sex: F= 4.6576, df=2, p=0.0097). Snails infected by trematode parasites also exhibited a significantly increased level of scarring due to attempted crab predation (Figure 11), (1-way ANOVA: F=33.2968, df=1, p < 0.0001).

Measurements for ranked height and width correlated significantly (Spearman's rank-correlation test: $R^2=0.867348$, $P=0.0000$, $F=7323.176$) indicating that both measures are an equivalent representation of shell size. Significant differences existed among shell height, site and depth (Figure 12), (ANOVA site vs height: F=184.5307, df=2, p<0.0001, ANOVA depth vs height: F=9.4042, df=3, p<0.0001, ANOVA site*depth vs height: F=2.6398, df=6, p=0.0151). Height of the smallest brooding *L. nassa* females varied across sites (ANOVA: F=112.7387, df=2, p<0.0001) and a significant interaction existed between depth and site (ANOVA depth*height: F=2.6536, df=6, p=0.0405) but no significant differences in height occurred among depths (ANOVA: F=1.3708, df=3, p=0.2756). Average height of the smallest three reproductive females at each site differed significantly between Jakobsen's and Hilltop (Figure 13), (Tukey-Kramer HSD: p<0.05).

Discussion

Parasite Prevalence and Diversity

As reported in previous studies, Hilltop Beach had significantly lower parasite prevalence but a higher parasite diversity in comparison to the two undisturbed sites at Jakobsen's Beach (Muthukrishnan 2004; McIntyre et al. 2005) The observed parasite prevalence at Hilltop may be due to sedimentation effects on the viability of trematode eggs and miracidium (McIntyre et al. 2005). Prior research found a higher rarified species richness of fish in disturbed areas (Alin et al. 1999), a possible explanation for the high diversity of trematodes at Hilltop Point. Since fish tend to be common second intermediate and/or definitive hosts in many trematode lifecycles, increased fish diversity could provide additional host options to support a greater number of trematode species. Hilltop also experienced a higher double infection frequency, consistent with the larger number of hosts and high trematode diversity. The double infection spatial results were not significant due to their infrequent occurrence, which is to be expected since larval trematodes in double infected hosts face the challenges of interspecific competition (Sousa 1993; Kuris and Lafferty 1994). In addition to a spatial scale variation, parasite prevalence was significantly lower at five meters than at the greater depths, suggesting that the second and definitive hosts are more abundant deeper than five meters (Figure 1).

Separating trematode morphotypes in these statistical analyses revealed that the morphotype F trematode appeared most consistently at deeper depths and morphotype E was more common in shallower depths (Figure 3). It is possible that the distribution of these different trematode morphotypes is based on the range of specific second intermediate and definitive hosts. In future studies, these trematode depth profiles could narrow possibilities for the next host in the trematode lifecycle. Parasite prevalence and identity may correspond with other species that commonly occur around the first intermediate host, *L. nassa*, providing candidates for future hosts.

Two of the five morphotypes recognized in *L. nassa*, E and G, encyst to fish. Morphotype E, (a presumed sanguinocolid), uses the fish for its second and definitive host, and morphotype G, pleurolophocercous cercariae, may use the fish as a second host (Schell 1970, pg. 25). Additional taxonomic work is underway to further identify the other unknown trematode morphotypes and their lifecycles.

Scarring, morphometrics and parasite prevalence

Scarring frequency due to attempted crab predation differed both among sites and between parasitized and uninfected individuals. Infected individuals experienced more scarring than uninfected ones, which corresponds with the prediction that crabs might be the second intermediate host for some trematode morphotypes. Crabs are likely candidates for morphotype F's second host. Several studies suggest co-evolutionary adaptations in the predator-prey relationships between crabs and *Lavigeria* gastropods (West and Cohen 1991, Rosales 2002, Marijnissen et al. unpublished results). *Platytylphusa armata* has a preferred depth range of 10-15 meters

(Marijnissen 2004), which matches the depth profile for morphotype F. The high correlation between scarring frequency and parasite prevalence may be an evolved response to decrease trematode traveling distance to a potential second host, the crab.

Variance throughout sites and depths indicates a general trend of patchiness in *L. nassa* morphological characteristics, particularly shell size. *Lavigeria nassa* populations in the Kigoma area are also characterized by high levels of genetic variation (Michel et al. 2005). Size differences between snails at Hilltop and Jakobsen's correlate inversely with sedimentation (McIntyre et al. 2005), but differences among depths and between the two Jakobsen's sites may be due to other genetic or environmental factors. The significant interaction between trematode parasite prevalence and height is likely due to the increased opportunity for parasite infection on a temporal scale. Gigantism and dwarfism are common host responses to trematode infection (Miura et al. 2006), yet do not appear to explain the increased size of parasitized individuals in *L. nassa* (Athuman 2004). Depth and site interactions in the height of smallest brooding females could be influenced by the small number of *L. nassa* collected deeper than 10m at Hilltop.

Preliminary observation of females experiencing early trematode infection indicates that broods may continue to develop after castration occurs. The broods of infected females were generally characterized by small numbers of late stage embryos, perhaps because the individuals already present in the brood develop normally despite the lack of continued egg production.

Conclusion and further study

Parasite prevalence differed by site and depth. These spatial variations are most likely due to the varying abundances of the specific second and final host. This study provided further clues to the likely second and final host candidates of the specific trematode morphotypes in *L. nassa*. Additional details will be released after further taxonomic work is completed on the specimens currently classified by morphotype.

Shell morphometric variables also differed across sites and depths, but initial analysis did not reveal a correlation between parasite prevalence and average shell size among populations. Scarring frequency correlated positively with parasite prevalence. Future work will focus on a potential relationship between brood size of *L. nassa* individuals and parasite prevalence and diversity.

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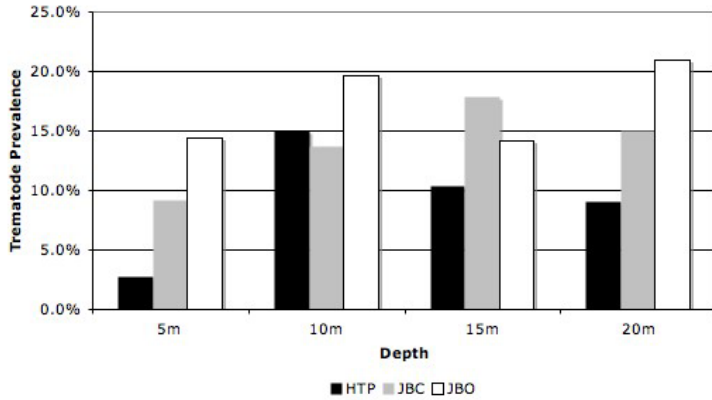


Figure 1: Trematode prevalence differed among depths and sites in *Lavigeria nassa*. Infection rates were significantly lower at Hilltop than either Jakobsen’s site.

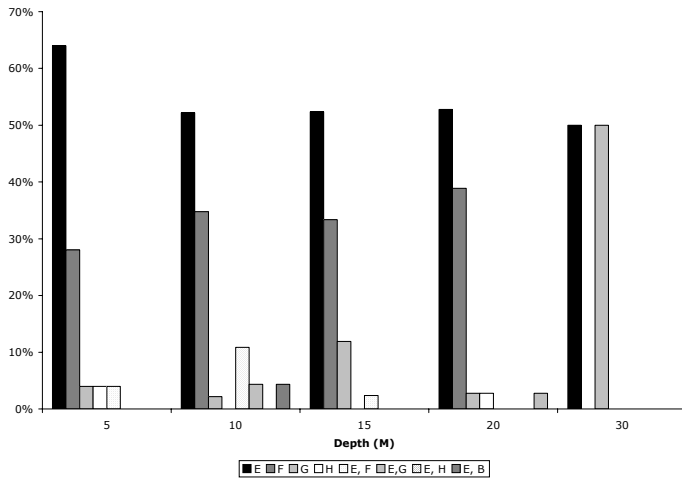


Figure 2: Trematode diversity varied significantly across sites, with Hilltop (HTP) displaying the highest parasite diversity. Trematode diversity did not differ significantly among depths.

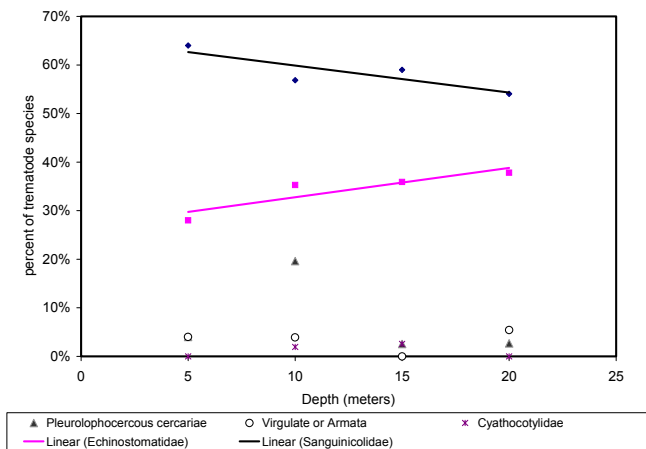


Figure 3: Although no significant differences existed between depth and trematode identity, morphotypes differed in their depth profiles. Morphotype F trended toward deeper waters ($R^2 = 0.814$) and the sanguinicolid morphotype trended toward shallower waters ($R^2 = 0.727$).

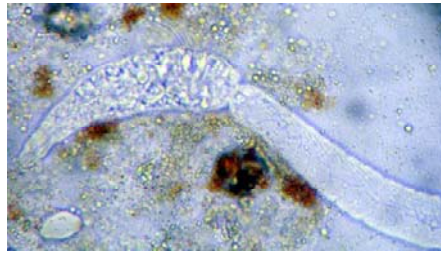


Figure 4: (E), with dorso-medial fanfold in view

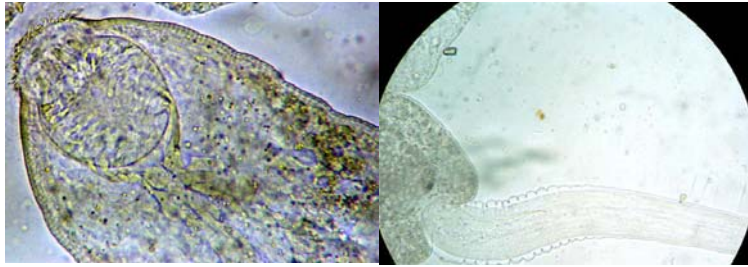
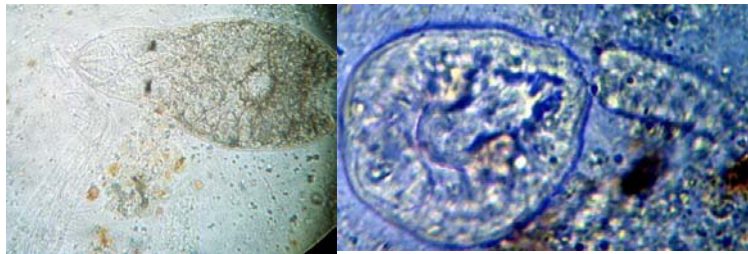
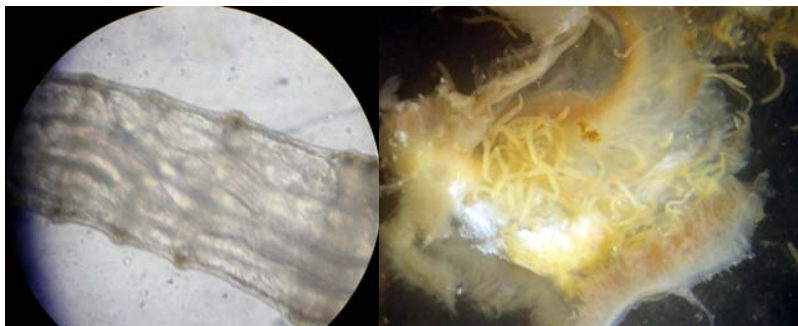


Figure 5: (F) with spiny collared oral sucker (left), and view of the tail (right)



Figures 6 and 7: Left: (G), with prominent eyespots, dorso-ventral fanfold and lateral finfold Right: (H), with a clear stylet in view.



Figures 8 and 9: Wormy type C, found in *P. damoni*, approximately 4mm in length, but can reach past 8mm (left) and wormy type C emerging from the rectum (right)

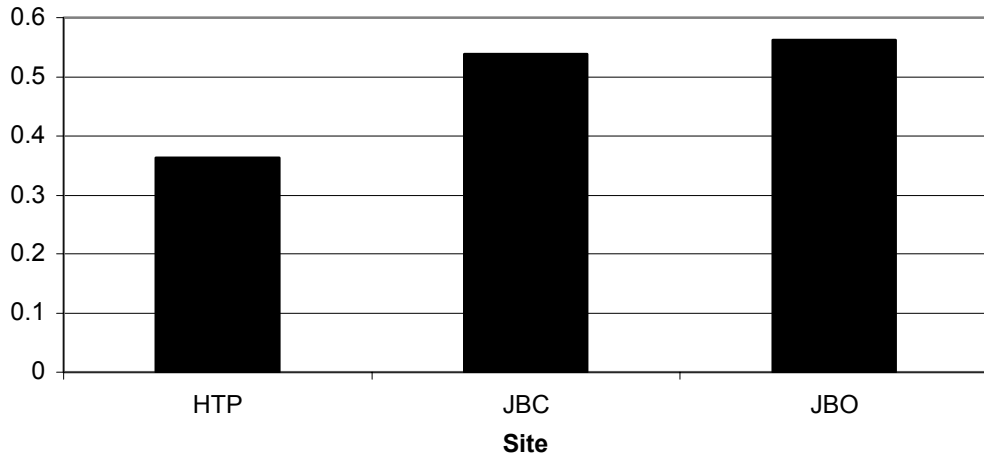


Figure 10: Scarring frequency of *L. nassa* due to attempted crab predation differed significantly among sites. *Lavigeria nassa* from Hilltop (HTP) had fewer scars than those from the two Jakobsen's sites.

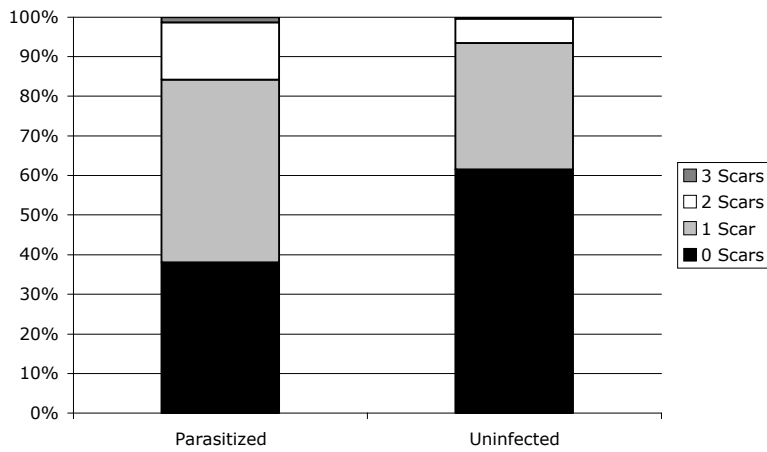


Figure 11: Parasitized *L. nassa* had significantly more scars from attempted crab predation than uninfected individuals.

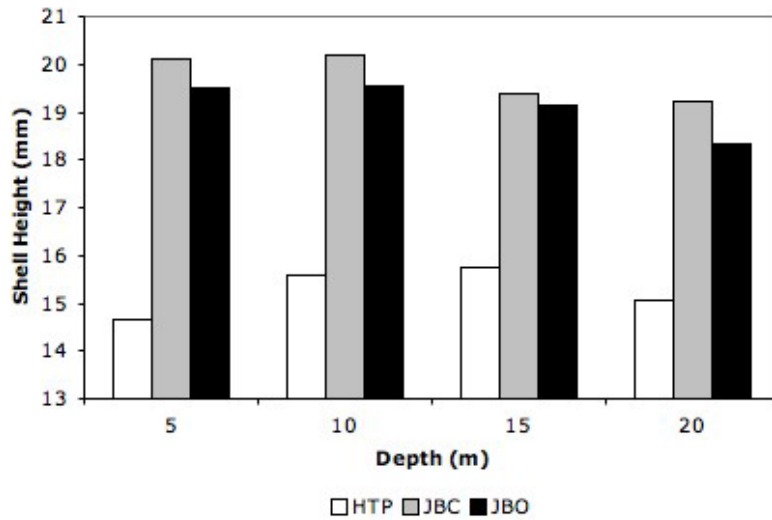


Figure 12: *Lavigieria nassa* shell height differed significantly across sites and depths. Individuals from Hilltop Point were significantly smaller than those from either Jakobsen's site at all depths.

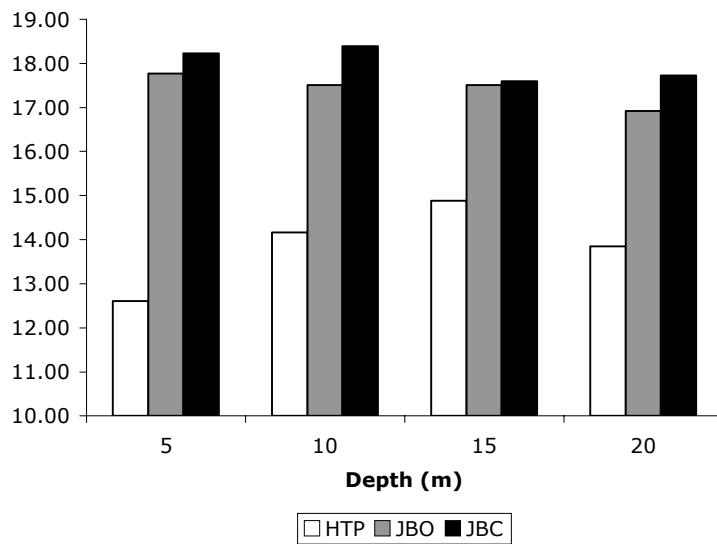


Figure 13: Size at maturity of *L. nassa*, represented by average shell height of the smallest three brooding females, differed significantly across sites and depths. The smallest brooding females from Hilltop were smaller than those from either Jakobsen's site.