Effect of age on the structure and activity of the pharynx of the free- living nematode Metarhabditis andrassyana (Rhabditidae)

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Research Article

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Abstract

Study of pharynx in a *Metarhabditis andrassyana* (Tahseen *et al*., 2004) revealed that the activity of pharynx declined with age and was also reduced in the absence of *E.coli*. A comparison between presence and absence of bacteria in the same age group revealed a reduction in both 2 day old individuals and 8 day old individuals. However, when nematodes were placed together in groups of varying numbers, pharyngeal pulsations declined as the numbers of specimens increased from 5 to 15 in the presence or absence of bacteria as a food source with age. In old worms, abnormalities were observed in the structure of the pharynx.

Introduction

The pharynx in nematodes connects the stoma or feeding apparatus with the intestine. Depending on the feeding habits of the nematodes the pharynx varies accordingly. It may be divided into two or more parts and is essentially muscular so that the food taken in can be forced into the intestine. In most nematodes it works as a pump to force the food into the intestine against internal turgor pressure (Harris & Crofton, 1957; Bennet-Clark, 1976; Avery & Shtonda, 2003). Pharyngeal pumping is accomplished by sequential contraction of muscle fibres that leads to wave of dilation which propagates along the lumen from anterior to posterior region (Fürst von Lieven, 2003). In most rhabditid nematodes the pharynx shows two basic activities: pumping and isthmus peristalsis. The pumping cycle that involves the corpus, anterior isthmus and terminal bulb begins by contraction of muscles that open the lumen in these regions. During isthmus peristalsis contraction of muscles pulls the lumen open in the posterior isthmus and transports the food into the terminal bulb (Doncaster, 1962; Seymour et al., 1983; Avery & Horvitz, 1989; Chiang et al., 2006). During the pumping phase the terminal bulb is isolated from the corpus and anterior isthmus because the posterior isthmus is closed (Avery & Shtonda, 2003). Peristalsis in the posterior isthmus is delayed and occurs after approximately every four pumps (Avery & Horvitz, 1987). Pharyngeal activity like the other body functions, declines as the nematodes age. This reduction in pharyngeal activity may be caused by several factors. In *C. elegans* ageing results in structural deterioration or sarcopenia of the pharynx muscles and is considered the primary cause of reduced pumping cycles (Johnson, 1987; Garigan et al., 2002; Herndon *et al*., 2002; Glenn et al., 2005). It was also confirmed that ageing produced no abnormal changes in the neurons of the pharynx (Herndon *et al*., 2002), suggesting that there was no nervous involvement in reduced pharyngeal activity. Laser ablation of identified neurons innervating the pharynx also revealed that, even in the absence of all pharyngeal neurons, pumping continues and only one pharyngeal neuron, M4, is essential for feeding and hence for life (Avery & Horvitz, 1987; Avery & Horvitz, 1989; Avery, 1993b).

Materials And Methods

Isolation of different age groups of males and females

Similar age groups of males and females of *M. andrassyana* were obtained by transferring developing J4 stage males and females on to separate NGM plates. The nematodes were harvested at the required age.

Feeding

Young nematode was placed on a strip of NGM agar 15 x 10 x 2 mm with bacteria *E.coli* on a glass slide. A coverslip was gently applied. The nematode was allowed to acclimatize for 5 min after which pharyngeal pumping were observed and photographed on an Olympus BX 50 DIC microscope.

Pharyngeal activity

Pharyngeal activity which typically has two phases – pumping and posterior isthmus peristalsis was studied in individual specimens and multiple specimens in groups of varying numbers.

i) Single specimens

Single specimens of two age groups were studied, 2 days old and 8 days old. Further, each set was observed in the presence and absence of the bacteria, *E.coli*. A strip of NGM agar 15 x 10 x 2 mm with or without bacteria was placed on a glass slide and a single individual was placed on it. A coverslip was gently applied. The nematode was allowed to acclimatize for 5 min after which pulsation and isthmus peristalsis were observed and recorded on a Zeiss Discovery V20 stereo microscope. There were twenty replicates using equal number of females and males for each set i.e. with bacteria and without bacteria. The observation time was two minutes. The same protocol was followed with the eight day old individuals.

ii) Multiple specimens

When multiple specimens in groups of varying numbers were used, only pharyngeal pulsation were observed. Groups of 5, 10 and 15 were studied. In each group two days old and eight days old specimens were observed separately. For both age groups in the three groups of nematodes pharyngeal pumping activity was recorded in the presence and absence of bacteria. All groups of nematodes and the ageing subgroups were studied on NGM agar plates and pumping were recorded for 2 minutes on a Zeiss Discovery V20 stereo microscope. There were ten replicates, five of males and five of females, for each set.

Pharynx structural changes

10–20 nematodes of two and eight day age groups were mounted individually on 2% agarose pads and paralyzed with alcohol (Wu *et al.*, 2019). The pharynx were studied and photographed using an Olympus BX 50 DIC microscope with a mounted ProgRes C3 camera.

Statistical analysis

All the data were statistically analyzed by one-way ANOVA using GraphPad Prism 7 to reveal significant difference between mean values of different age groups. All values are presented as mean ± deviation

(SD). The probability levels of 0.05 were considered statistically significant.

Results

Feeding

The bacterial food source is ingested via the stoma. The lumen of the pharyngeal tube communicates with that of stoma, even when the rest of the pharyngeal lumen is closed. The anterior end of the pharynx contains the corpus, which brings about ingestion during muscle contraction. The posterior end of the pharynx contains the terminal bulb, crushes the bacteria between the grinder so they can be digested and in the intestine. Pharyngeal muscle contraction opens the lumen and relaxation closes it. During pumping, it is necessary for different parts of the pharynx to contract to effectively transport food to the intestine. A pharyngeal pump begins with the nearly simultaneous contraction of the corpus and the terminal bulb resulting in the drawing of food particles into the pharyngeal lumen. This is followed by a contraction of the anterior isthmus. The anterior part of the corpus relaxes first, then the posterior part of corpus which is adjacent to the isthmus. The bacteria ingested are then trapped in the anterior isthmus. Posteriorly-propagating contractions of the posterior isthmus, known as peristalsis, transport bacteria from the anterior isthmus to the terminal bulb. Peristalsis in the posterior isthmus occurs every 4–5 pumping cycles (Fig. 1). In the basal bulb, at the beginning of grinding process, the open passage between terminal bulb and intestine results in a regurgitation of food into the grinder (Fürst von Lieven, 2003). The contraction of the lumen of terminal bulb pushes particles out of the haustrulum and into the intestine.

Pharyngeal activity

i) Single specimens

Pharyngeal activity declined with age and was also reduced in the absence of *E.coli*. Pumping activity declined significantly ($p < 0.05$) by 68% and isthmus peristalsis by 73% in eight day old nematodes as compared to two day old, in the presence of bacteria. In the absence of the food source the decline was even greater with pumping reduced by 73% and peristalsis by 78%. A comparison between presence and absence of bacteria in the same age group revealed a reduction of 27% for pumping as well as peristalsis in 2 day old individuals and 39% in 8 day old individuals (Fig. 2). All declines were significant ($p < 0.05$) $F(3, 72) = 4621$ $p < 0.0001$. The average number of pulsations per peristalsis remained relatively constant between 4 and 5. The average for two day individuals was 4.1 with and without bacteria while in eight day individuals 5.0 with bacteria and 4.7 without bacteria.

ii) Multiple specimens

When nematodes were placed together in groups of varying numbers, pharyngeal pulsations declined as the numbers of specimens increased from 5 to 15 in the presence or absence of bacteria as a food source in two day old nematodes (Fig. 3). In eight day old nematodes, pumping activity was greatly reduced as compared to two day old nematodes and it declined further with increase in numbers both with and without a food source. In two day old nematodes in the presence of *E. coli* the decline in pumping rate of the fifteen nematode group was significant at 17% as compared to the five nematode group. In the absence of bacteria, the difference between the said two groups was even greater (29%). In eight day old nematodes the reduction in pulsations was significant ($p < 0.05$) $F (6, 108) = 44.98$ $p < 0.0001$at 26% in the presence of bacteria and only 9% in absence of bacteria between five and fifteen nematode groups (Fig. 3). In all two day old nematode groups pharyngeal pumping was significantly higher ($p < 0.05$) in the presence of bacteria than in its absence. The reduction varied from 27% in the five nematode group to 38% in the fifteen nematode group. In the eight day group significant reduction of 22% and 26% occurred between five and ten nematode group and was only 4% in the fifteen nematode group (Fig. 4A). In the presence of bacteria when groups of similar numbers were compared between young and old individuals then there was a drop in pulsation rate from 71% in the ten nematode group to 75% in the fifteen nematode group. Without food the pumping rate was reduced to 61% in the fifteen nematode group, 69% in the ten and 70% in 5 nematode groups (Fig. 4B).

Pharynx structural changes

With age several structural changes occurred on the surface of the pharynx as well as in the lumen, tissues and the grinder. Shrinkage and distortion appeared on the outer lining on some parts of the procorpus (Fig. 5C) and the basal region of the basal bulb (Fig. 5D). The lining becomes thinner and depressed on the procorpus and appears to shrink inwards in the basal bulb distorting the smooth and uniform outline. The distorted outline of the basal bulb is also visible in Fig. 5 (E, F). The internal changes were generally more pronounced. In some specimens the metastegostomal region became swollen with a large vacuolated appearance (Fig. 5A). Vacuolated areas also appeared in the corpus (Fig. 5C) and basal bulb (Fig. 5E). Vacuolated areas showed a hyaline structure and appeared to be filled with fluid. There was no sign of muscular tissues in the vacuoles suggesting that it may have been reabsorbed. In addition, the procorpus lumen often became distorted and irregular (Fig. 5B). The basal bulb occasionally became elongate with a disorganized grinder (Fig. 5G). In many nematodes the haustrulum muscles near the base of the bulb was difficult to visualize indicating degenerative changes (Fig. 5F).

Discussion

In most rhabditid nematodes, pharyngeal contractions open the lumen and draws food in while relaxation closes the lumen and traps and transports the food down towards the posterior part of the pharynx. The contraction and relaxation cycles in *M. andrassyana* is almost identical to that observed in *C. elegans* (Avery & Shtonda, 2003). A slight delay in the relaxation of the anterior isthmus makes it possible to receive and accumulate the bacteria that have passed down from the anterior corpus. Like in *C. elegans* (Seymour et al., 1983; Avery & Shtonda, 2003) bacteria tend to accumulate at the base of the stoma before entering the pharyngeal lumen at the end of the contraction cycle. Regurgitation of intestinal contents into the basal bulb was not observed as in several rhabditid nematodes (Fürst von Lieven, 2003) but the possibility of this happening may not be ruled out.

M. andrassyana showed the general tendency of decline in the pharyngeal pulsations with age when studied singly. Similar observations have also been made in *C. elegans* (Bolanowski et al., 1981; Kenyon et al., 1993; Huang et al., 2004). Besides age, availability of food also appeared to influence the pharyngeal pumping as within the same age group pulsations declined significantly in the absence of bacteria. Croll & Smith (1978) and Horvitz et al. (1982) suggested that bacteria stimulated pharyngeal pulsation, a phenomenon that may also be operating in *M. andrassyana*. When nematodes were studied in groups, it becomes clear that numbers also influence the rate of pulsations in the presence of food or without food. In two day old nematodes the decline was significant between the three groups. In the absence of food although pulsations were significantly less than those with food, the differences between the three groups varied significantly. In the eight day old nematodes pulsations differed significantly only between two group. The rate of decline in the absence of food came down to just 4% in the fifteen nematode group while it was maintained above 20% in the other two groups. This near similarity in the rate of pulsations in the absence of food in the fifteen nematode group may represent a basal rate, just sufficient for survival. In young 2 day old nematodes, the significant decline in pharyngeal pumping in the presence of bacteria perhaps indicates that secretions of the nematodes together with excrements and other metabolic waste products may also be playing a role in the pulsation rate via chemo-sensory pathway. If contact with one another is also considered as a probable cause, it may represent a thigmotactic response affecting the several pathway of pharyngeal pumping.

Correlated with pharyngeal pulsation and synchronous with its decline in ageing individuals is the gradual degenerative changes in the structure of the pharynx. The swelling and vacuolation of the corpus adjacent the metastegostom not only deforms the stegostom but in all probability also affects the process of ingestion and may be one cause of bacteria clogging the stoma. The distortion of the luminal lining, a slight shrinkage/narrowing of the corpus wall and the vacuolation in the basal region of the corpus could affect the transport of food down the pharynx. All these changes in the anterior pharynx may not affect pulsations *per sec* but could certainly influence ingestion via metastegostomal disorganization and transport because of luminal distortion and vacuolation of corpus tissue and could result in accumulation of bacteria in the pharynx. Chow et al. (2006) suggested that bacterial plugging was a consequence of reduced pumping and not the cause as suggested by Garigan et al. (2002). Our observations of the deteriorative changes in the basal bulb reveals an involvement of almost all region of the bulb- the outer surface, muscles and the grinder. The most spectacular changes are in the muscles in all parts of the bulb that may severely impair the pumping mechanism. Further the distortion of the grinder, brought about by muscle degeneration or mechanical damage caused by continuous pumping may also be a cause of bacterial plugging of the basal bulb.

Declarations

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ETHICAL STATEMENT:

1. There was no need for ethical approval for using nematode as model.
2. Author has no conflict of interest.
3. First author conducted the experiment work and writing the manuscript while other author help to develop the study design and manuscript editing.

References

1. Anton, A.H., Berk, A.I. & Nicholls, C.H. (1992). The anesthetic effect of alcohols and alkanes in *Caenorhabditis elegans. Research communications in chemical pathology andpharmacology*78:69-83.
2. Avery, L. (1993). Motor neuron M3 controls pharyngeal muscle relaxation timing in *Caenorhabditis elegans*. *Journal of Experimental Biology***175**:283–297.
3. Avery, L. & Horvitz, H.R. (1987). A cell that dies during wildtype *elegans* development can function as a neuron in a *ced-3*mutant. *Cell* **51**:1071–1078.
4. Avery, L. & Horvitz, H.R. (1989). Pharyngeal pumping continues after laser killing of the pharyngeal nervous system of *elegans*. *Neuron***3**: 473–485.
5. Avery, L. & Shtonda, B.B. (2003). Food transport in the *elegans* pharynx. *Journal of Experimental Biology*. **206**: 2441-2457. DOI: 10.1242/jeb.00433
6. Bennet-Clark, H.C. (1976). Mechanics of nematode feeding. In: the Organization of Nematodes (N.A. Croll, ed.). Academic Press, London, New York, San Francisco.pp. 313-342.
7. Bolanowski, M.A., Russell, R.L. & Jacobson, L. A. (1981). Quantitative measures of ageing in the nematode *elegans*. I. Population and longitudinal studies of two behavioural parameters. *Mechanisms of Ageing and Development*. **15(3)**: 279-295.
8. Chiang, J.T., Steciuk, M., Shtonda, B., & Avery, L. (2006). Evolution of pharyngeal behaviors and neuronal functions in free-living soil nematodes. *Journal of Experimental Biology****209****:*1859-1873.
9. Chow, D.K., Glenn, C.F., Johnston, J.L., Goldberg, I.G. & Wolkow, C.A. (2006). Sarcopenia in the *Caenorhabditis elegans*pharynx correlates with muscle contraction rate over lifespan. *Experimental Gerontology* **41(3):** 252-260.
10. Croll, N.A. & Smith, J.M. (1978). Integrated behavior in the feeding phase of *Caenorhabditis elegans* (Nematoda). *Journal Zoology*. (London) **184**: 507–517. DOI: 10.1111/j.1469- 7978.1978.tb03304x

11. Doncaster, C.C. (1962). Nematode feeding mechanisms. I. Observations on *Rhabditis*and *Pelodera*. *Nematologica* **8**: 313–320.
12. Duhon, S.A. & Johnson, T.E. (1995). Movement as an index of vitality: Comparing wild type and the *age-1*mutant of *Caenorhabditis elegans.Journal Gerontology* **50(5)**: B254-261. DOI: 1093/gerona/*50A*.5
13. Fürst Von Lieven, A. (2003). Functional morphology and evolutionary origin of the three part pharynx in nematodes. *Zoology***106**: 183-201.
14. Garigan, D., Hsu, A.-L., Graser, A.G., Kamath, R.S., Ahringer, J. & Kenyon, C., (2002).Genetic analysis of tissue aging in *Caenorhabditis elegans*: a role for heat-shock factor and bacterial proliferation. **161(3):** 1101–1112.
15. Glenn, C.F.,Chow, D.K., David, L., Cooke, C.A., Gami, M.S., Iser, W.B., Hanselman, K.B., Goldberg, I.G. & Wolkow, C.A. (2005).Behavioral deficitsduringearly stages of aging in*Caenorhabditis elegans* result from locomotory deficits possibly linked to muscle frailty. *The journals of gerontology. Series A,Biological sciences and medical sciences* **59**: 1251-1260.
16. Harris, J.E. & Crofton, H.D. (1957). Structure and function in the nematodes: Internal pressure and cuticular structure in *Ascaris*. *Journal of Experimental Biology* **34**: 116-130.
17. Herndon, L.A., Schmeissner, P.J., Dudaronek, J.M., Brown, P.A., Listner, K.M., Sakano, Y., Paupard, M.C., Hall, D.H. & Driscoll, M. (2002). Stochastic and genetic factors influence tissue-specific decline in ageing *elegans*. *Nature***. 419(6909):**808-814. DOI:10.1038/nature01135
18. Horvitz, H.R., Chalfie, M., Trent, C. & Sulston, J.E., (1982). Serotonin and Octoamine in the nematode *Caenorhabditis elegans*. **216(4549)**: 1012-1014. DOI:10.1126/science.6805073
19. Huang, C., Xiong, C.&Kornfeld, K. (2004). Measurements of age-related changes of physiological processes that predict lifespan of *Caenorhabditis elegans*. *Proceeding National Academy Science USA.***101(21):** 8084–8089. DOI:1073/pnas.0400848101
20. Johnson, T.E. (1987). Aging can be genetically dissected into component processes using long-lived lines of *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences* **84**: 3777-3781.
21. Kenyon, C., Chang, J., Gensch, E., Rudner, A.&Tabtiang, R.A. (1993). *elegans*mutant that lives twice as long as wild type. *Nature*. **366(6454)**: 461–464. DOI: 10.1038/366461a0
22. Seymour, M., Wright, K.A. &Doncaster, C.C. (1983). The action of the anterior feeding apparatus of *Caenorhabditis elegans*(Nematoda: Rhabditida). *Journal of Zoology London.* **201**:527–539.
23. Zhong-Qin, Wu., Li., Jin-Kui, Ma.& Zong-Jun, Li. (2019). Effects of ethanol intake on anti-oxidant responses and the lifespan of *Caenorhabditis elegans*. *CYTA- Journal of Food.***17**: 288-296.

Figures

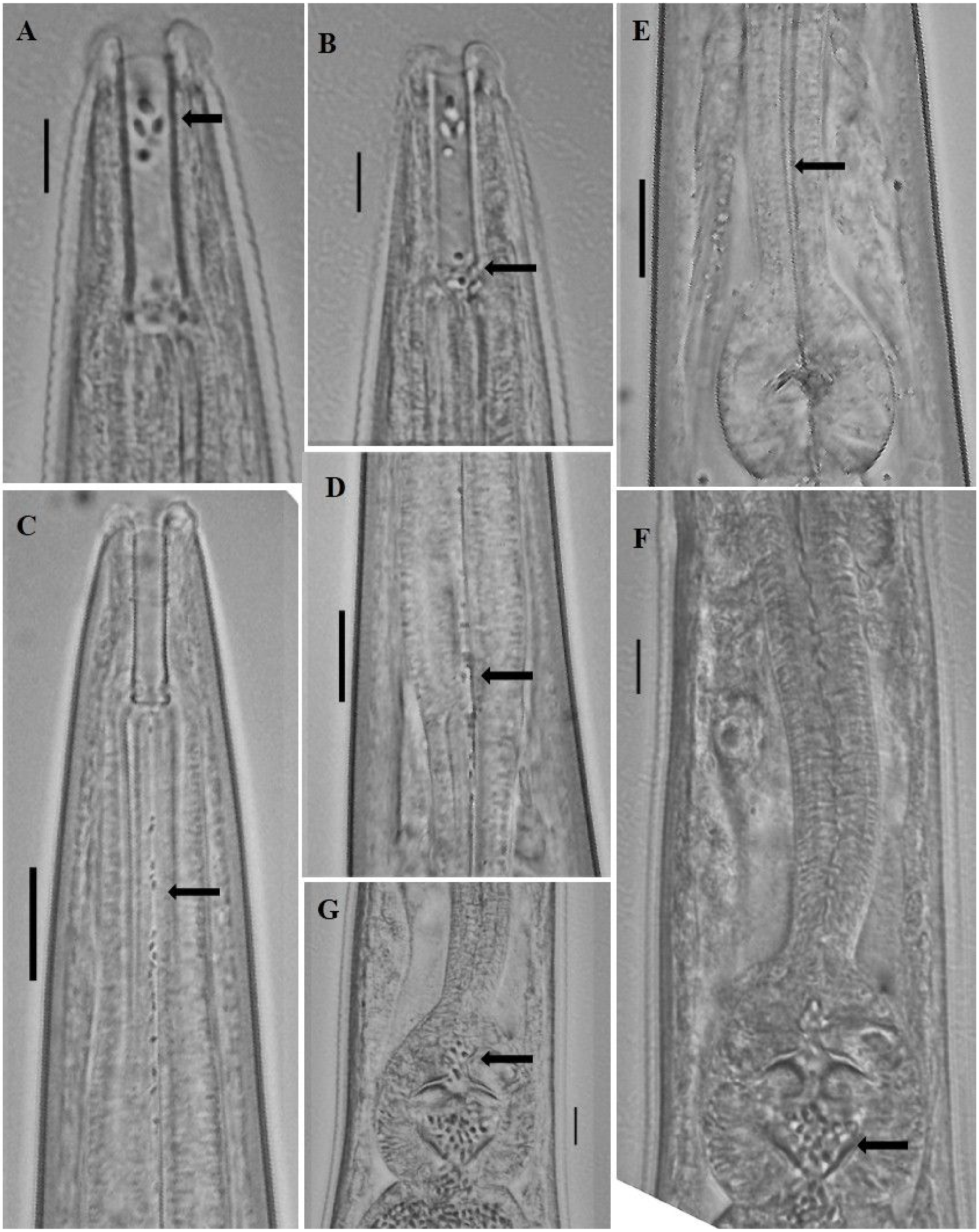

Figure 1

Movement of food in the pharynx. A & B. Food particle in stoma, C. Corpus region, D. Anterior isthmus, E. Posterior isthmus, F & G. Basal bulb & Intestine. (Arrow indicate the position of bacteria). Scale bar: A-G = 10 μm.

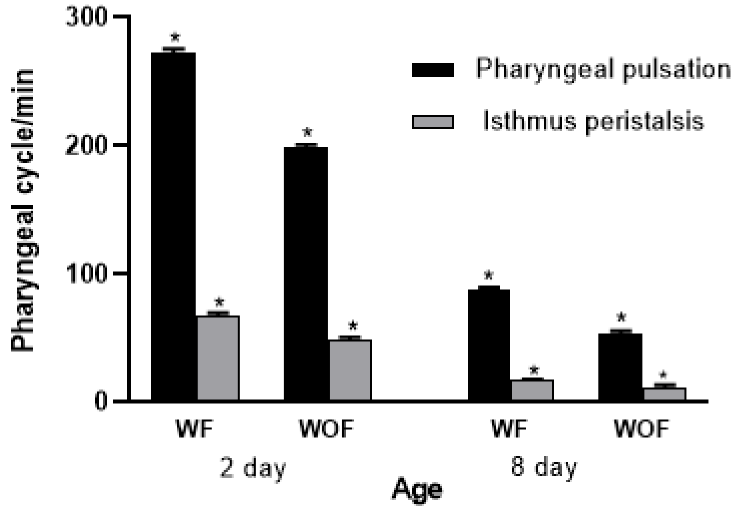

Figure 2

Pharyngeal activity in different age groups of *M. andrassyana* in the presence (WF) and absence of *E.coli* (WOF). Significant differences are indicated by a single asterisk (P < 0.05).

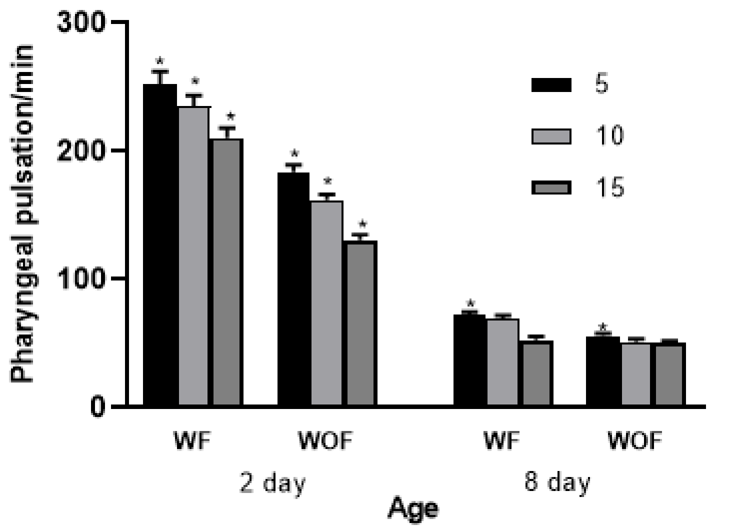

Figure 3

Pharyngeal pumping in two age groups of varying numbers of *M. andrassyana* in thepresence (WF) and absence of food (WOF). Significant differences are indicated by asingle asterisk (P < 0.05).

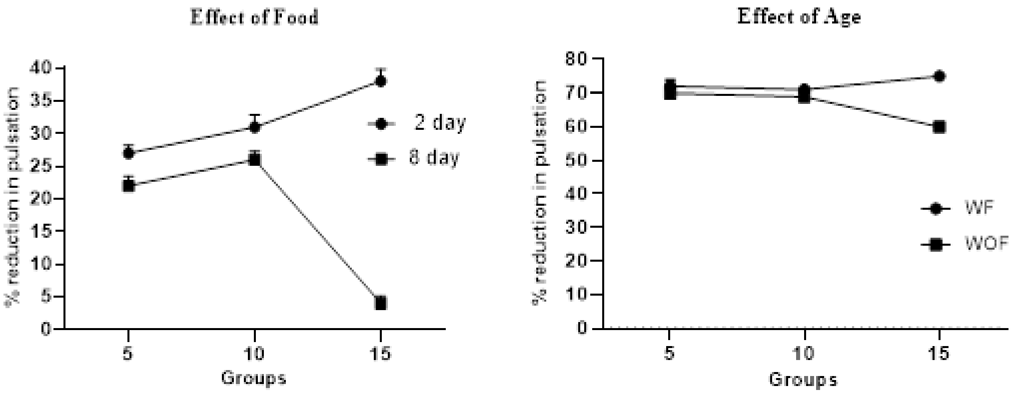

Figure 4

Pharyngeal pumping rates of different groups of individuals. A) % reduction in pulsation in the absence of food. B) % reduction in pulsation with age.

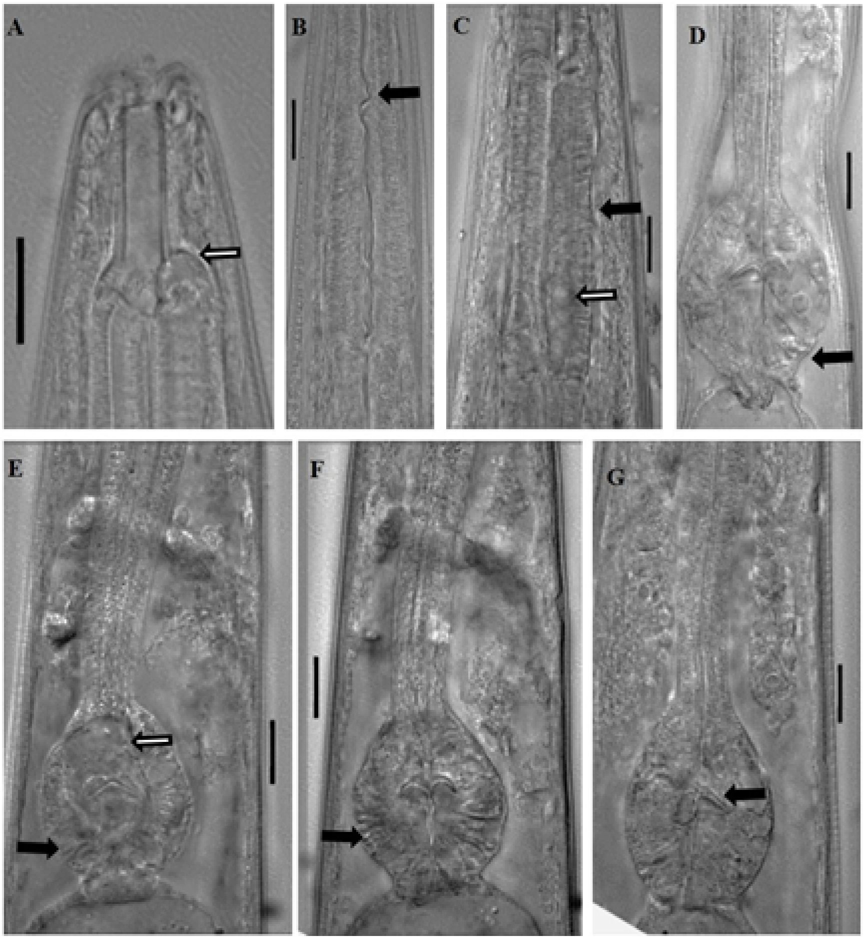

Fig 5: Pharynx of aged nematodes; A. Vacuole at the base of stoma in older worms; B. Irregular lumen of pharynx in ageing; C. Degeneration in corpus (➡) and vacuoles present (⇨; D. Irregular basal bulb wall (➡); E. Large vacuole in basal bulb (⇨ and muscles degeneration (➡ F. Degenerated muscle at the region of basal bulb (➡; G. Structure of grinder changed and became asymmetrical (➡.Scale bar: A-G = 10 µm

Figure 5

See image above for figure legend.