

Effect of Age on Pharyngeal Pumping in Two Species of Free-Living Nematodes

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ABSTRACT: Pharynx is a neuromuscular pump present at the anterior end of the alimentary tract. Feeding rate as well as the precise timing of pharyngeal movements, is required for efficient feeding and for survival in nature. Described here are the age-related changes in pharyngeal behaviour of two rhabditid nematodes viz., *Metarhabditis andrassyna* and *Teratorhabditis palmarum*. In both the species the average pharyngeal pulsation were more in the presence of bacteria than in its absence. Pulsation rates declined with age in both species in both the conditions. Deterioration in the structure of the basal bulb was also observed.

Keywords: Age, behavior, pharynx, rhabditid.

A bacteriovorous nematode consumes bacteria and its feeding apparatus, the pharynx, is neuromuscular organ that functions as a pump (Avery *et al.*, 2003). The cycle of contraction and relaxation that draws food from the environment and filters bacteria from liquid is referred to as pharyngeal pumping. Old adults pump and defecate more slowly as compared to young adults (Bolanowski *et al.* 1981; Kenyon *et al.* 1993; Duhon & Johnson 1995). Feeding motions are monitored by the presence of food in the nematodes environment. The rate of pumping decides the amount of food intake and the rate of growth. The overall structure and function of the pharynx muscles can be easily monitored under a light microscope. Garigan *et al.* (2002) and Herndon *et al.* (2002) showed that ageing is also associated with structural deterioration of the pharynx muscles. Hence the pharynx suffers both structural and functional declines. The main function of pharynx is to pump food against the internal body pressure and prevent gut contents from being regurgitated (Bennet-Clark, 1976). The basic mechanism of pharyngeal pumping is accomplished by sequential contraction of muscle fibres (striated) that leads to wave of dilation which propagates along the lumen from anterior to posterior region (Lieven, 2003). We studied the pharyngeal pumping and structural changes in the basal bulb.

MATERIAL AND METHODS

Metarhabditis andrassyna and *Teratorhabditis palmarum* were cultivated on NGM agar plates with the *Escherichia coli* strain OP50 following standard protocols (Brenner, 1974).

Pharyngeal pumping: Pumping assays were performed on agar plates at room temperature using a Zeiss Discovery V20 Stereomicroscope. Pumping rate was determined as the number of contractions and relaxations in the grinder of terminal bulb in one minute. For this purpose, two sets of NGM plates were prepared. One set of plates were inoculated with *E. coli* OP50 and another set was maintained without *E. coli*. Nematodes of different age groups were selected i.e., 2, 6 and 10 days old and placed separately on both sets of dishes with or without *E. coli* OP50. Twenty replicates were maintained to ensure accuracy. The nematodes were allowed to acclimatize for ten minutes and then the number of pumping were observed over a two minute period. From this, average pumping rate was calculated. If no pumping was seen in two minute, the worm was recorded as non-pumping.

Metacarpus structure: 10-20 individual nematodes of each age were mounted on 2% agarose pads and paralyzed with alcohol. The pharynx was studied and photographed using an Olympus BX 40 DIC microscope with aProgres C3 camera mounted.

Data 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 \pm deviation (SD). The probability levels of 0.01 were considered statistically significant.

RESULTS

Pharyngeal pumping declined gradually with age in the presence or absence of bacteria. When bacteria were absent, pumping rate was significantly lower as compared to when bacteria were present in all age groups of both species. In *M. andrassyna* pharyngeal contraction declined from 268 ± 26.4 pumps per min in 2 days old reduced to 88 ± 7.63 pumps per min in 10 days old in the presence of bacteria. In the absence of bacteria pulsation declined from 199 ± 15.4 in 2 days old to 56 ± 11.2 in 10 days old individuals. Similarly in *T. palmarum* contractions declined from 248 ± 16.20 pumps per min to 108 ± 9.14 pumps per min in 2 & 10 days old. Here also in the absence of bacteria pulsation declined from 196 ± 13.6 in 2 days old to 86 ± 9.5 in 10 days old individuals (Fig. 1). Among the two genera, rate of pulsation was found to be high in *T. palmarum*. It was found that muscles weakening during aging resulted in bends in the length of isthmus (Fig. 2A, B & C). In some cases, the muscle swelled due to either bacterial infection at old stage (Fig. 2F) and in most other cases they shrunk, distorting the overall look (Fig. 2C & F). The surface morphology of the basal bulb was smooth in young adults (Fig. 3A & D) as compared to an irregular and rugged appearance in aged worms (Fig. 3C & F). The shape also appeared to change being rounded and rhomboidal in young individuals (3A) and flattened-oval (3C) in old worms. In aging adults, muscle cells at the basal region appeared deformed and shrunken that lead to surface deterioration (Fig. 3H) as compared to younger worms (Fig. 3G). It was also observed that feeding ability was

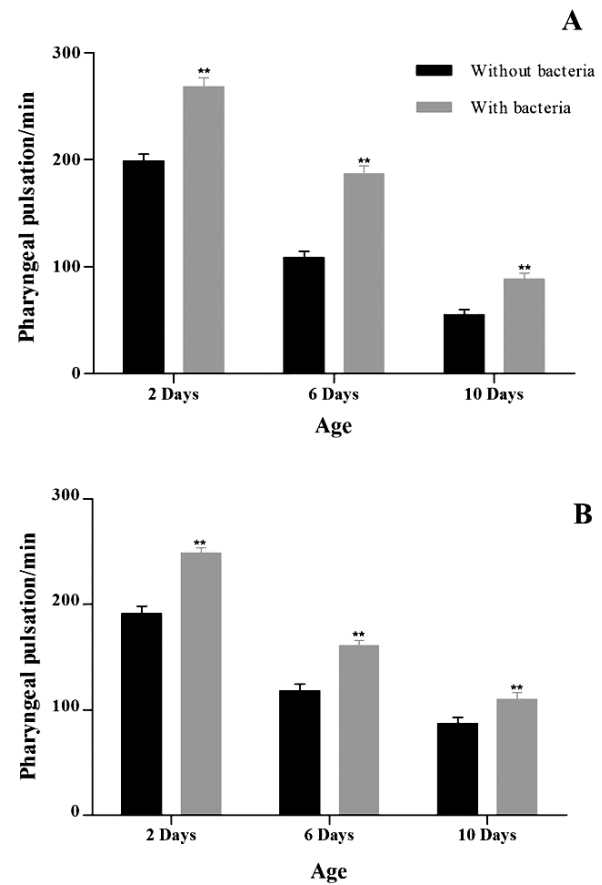


Fig. 1. Pharyngeal pumping rates of different age groups of *M. andrassyna* (A) and *T. palmarum* (B)

higher in younger worms that lead to bacterial clogging in grinder region (Fig. 2D & E) while due to weak musculature of grinder in older worms may also lead to bacteria clogging (Fig. 3I). In both cases it would lead to slower pumping rate.

DISCUSSION

The decline in the rate of pharyngeal pumping with age in *M. andrassyna* and *T. palmarum* is consistent with earlier observations on *C. elegans* (Kenyon *et al.*, 1993). While Croll and Smith (1978) and Horvitz *et al.*, (1982) also observed that the feed-bacteria stimulated the pumping activity, it was also observed in both nematodes that the rate of pumping declined with or without food. In young adults of *C. elegans*, the pharynx pumps, approximately 200-300 times/min and this pumping

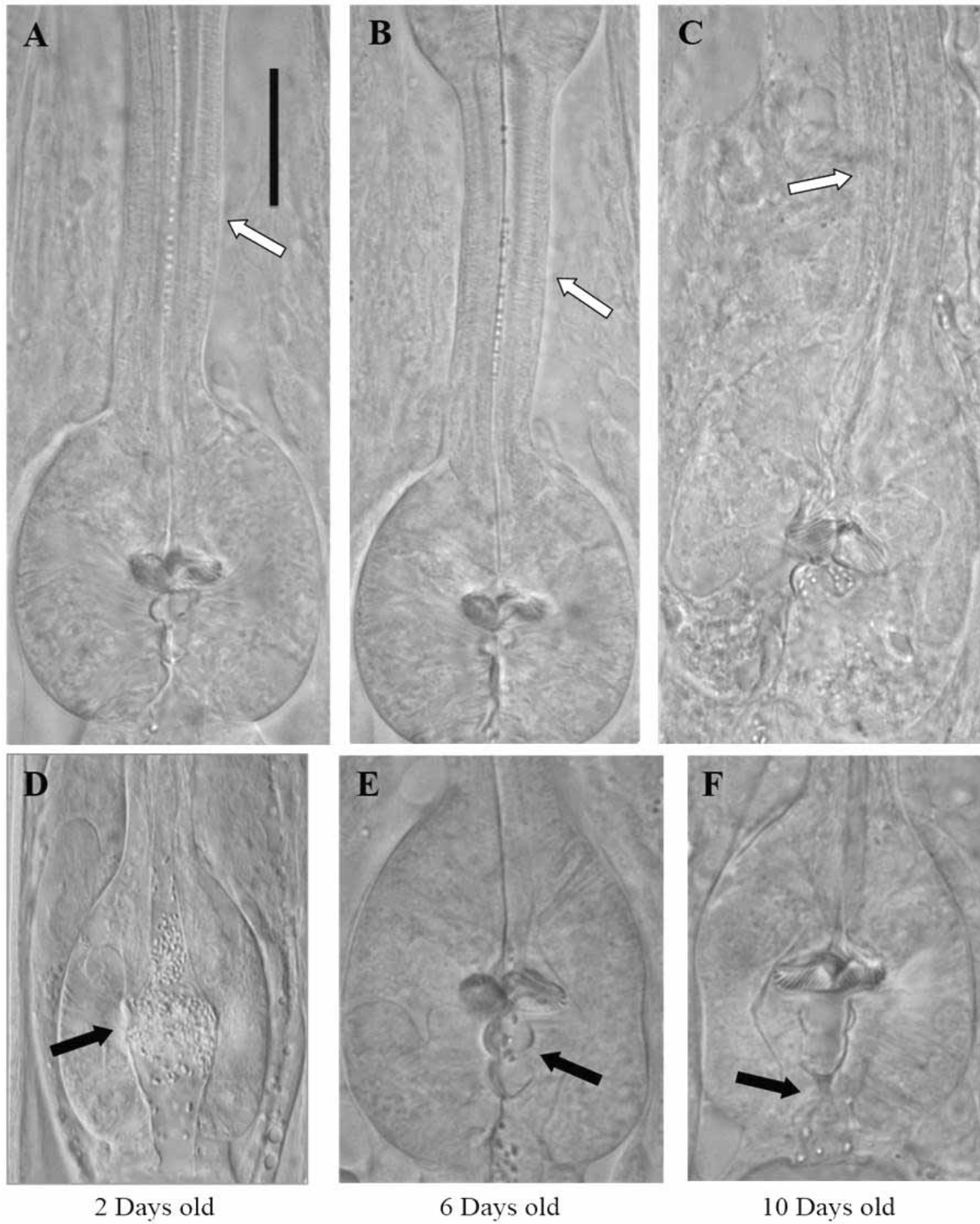


Fig. 2. Showing bend in isthmus length with age (A, B & C). Bacterial clogging in basal bulb of aging nematode (D, E& F). Scale bar: A, B, C, D, E & F = 20 μ m. (Black arrow shows bacterial clumping & White arrow represent bend in isthmus).

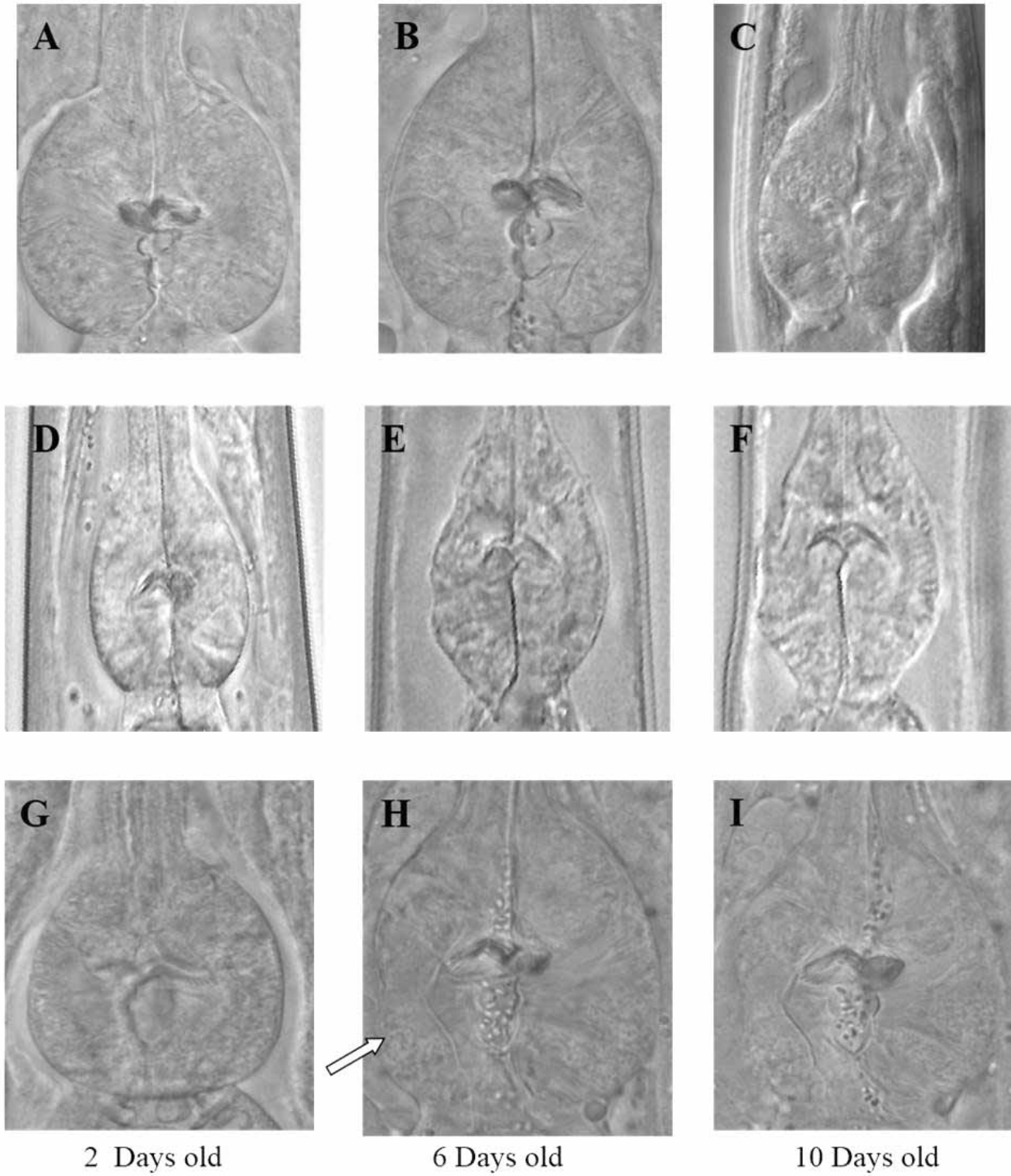


Fig. 3. Structural and morphological observation in different age nematodes (A, B, C, G, H & I) in *T.palmarum* and (D, E & F) in *M.andrassyana*. Scale bar: A, B, C, D, E, F, G, H & I = 20 μ m. (White arrow show surface breakdown).

rate declined gradually with aging (Bolanowski *et al.*, 1981; Huang *et al.*, 2004). Similarly, we also observed progressive rate of decline in both nematodes species. The rate of muscle contraction appeared to be correlated with structural and functional aspects of pharynx.

The damage in large muscles cells of the basal bulb was observed and isthmus appeared bent in old individuals of *T. palmarum*. The muscles weaken and hence rate of muscles contraction decreases. In mutants of *C. elegans* with slower pharyngeal pumping rates the muscle structure was better preserved (Chow *et al.*, 2006). However, structural decline was not significantly delayed during aging in slow pumping nematodes, perhaps indicating a diagnostic effect in the progression of muscle damage. Age-related pharyngeal functional decline is most likely intrinsic to the pharynx and not due solely to microbial invasion or toxicity (Chow *et al.*, 2006; Zhao *et al.*, 2017). Moreover, one of the possible reasons of swollen pharynx (pharyngeal muscle near the grinder) was due to bacterial accumulation in that region. Normal pharynx contained either no invading *E. coli* or small, membrane-bound bacterial inclusions usually near the grinder region. Further, muscle contraction in the weakened tissue may cause it to deteriorate. We believe that contraction-related injuries are an accessible factor only during young and middle age, when the muscles contract actively. Beyond this phase, further impairment of muscle contraction may be compounded by other physiological factors, viz, the nutritional state of the individual or impaired nervous stimulation or retarded physical mobility.

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