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Epitoky in polychaetes: when benthic worms go pelagic

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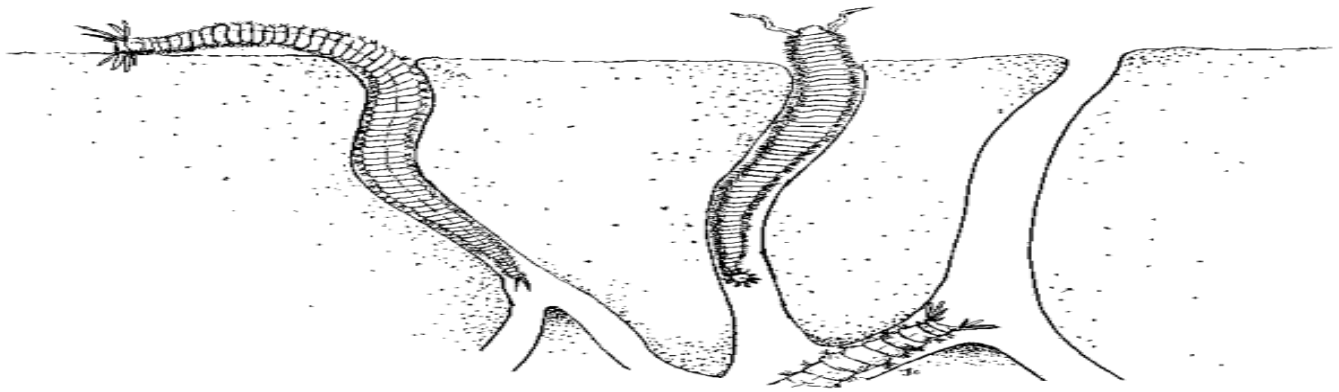

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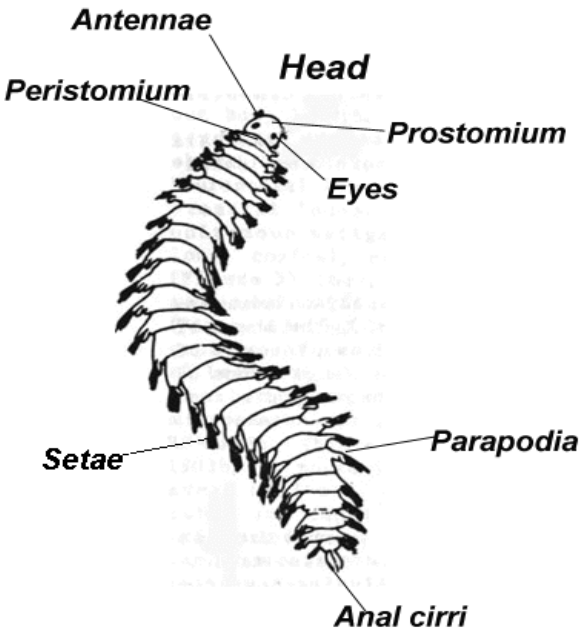
INTRODUCTION

Polychaetes, also known as bristle worms, are multi-segmented worms of the class of annelids and with more than 10.000 described species. Most species are found along intertidal areas, especially on sandy or muddy beaches. The majority of the polychaetes are benthic, but there are fifty planktonic species distributed throughout the world as well.
A major morphological characteristic of polychaetes are the parapodia. Each body segment contains a pair of these fleshy appendages, which in their turn have many bristles (setae) embedded. The setae come in many shapes and are used for movement. The head of a polychaete bears short sensory projections, two pairs of eyes, several tentacles and three antennae. Polychaetes grow by adding segments just ahead of the pygidium (tail). Their size ranges from less than 2,5 cm up to 3 m long.



Polychaete morphology

Polychaetes have many reproduction strategies, including external brooding, viviparity and hermaphrodism. These animals can be semelparous (reproducing only once during their life) or iteroparous (reproducing more than one time during their life). Before reproducing, most species undergo morphological and physiological modifications when they become sexually mature. This sexual transformation is known as epitoky: atokous (juvenile) polychaetes transform and become epitokous (sexually mature). Epitoky prepares the worms for a brief pelagic existence and improves the chances that sexual partners will find each other (Clark, 1961 in Chatelain et al, 2007; Schöttler, 1989; Fischer, 1999).

Malmgren (1867) was the first zoologist to suggest the existence of epitoky and later Ehlers (1868) demonstrated this phenomenon in the classic case of epitokous nereids, errant polychaetes that live in mucous-lined burrows in or near low tide. All species from this family are semelparous and most are epitokous. In this paper, four aspects of epitoky in polychaetes are at interest, leading to the following four main questions:

1. Epitokous transformation is a process in which new tissues differentiate and some old tissues degenerate or transdifferentiate (Fischer, 1999). Which morphological changes do polychaetes undergo in the process of epitoky and why are these changes necessary?

2. Resources are used up by the transforming body so that the biochemistry and the transfer kinetics of metabolites can be studied (Fischer & Hoeger, 1993; Fischer et al., 1996 in Fischer, 1999). What physical changes do the polychaetes undergo to prepare them for a short pelagic lifestyle and why are these changes necessary?

3. Epitokous transformation is synchronized with gametogenesis, and may seem subject to very precise timing by external parameters e.g. by the lunar cycle (Hauenschild, 1956). Does moonlight trigger the process of epitoky?

4. Epitokes assume a pelagic style of life (Fischer, 1999). Why going pelagic?

1. Morphological transformation

The epitokous transformations of the tissues have been studied in most detail in the nereidids (Claparède, 1870 in Fischer, 1999). For this group, tissue differentiation has been described in producing new sets of setae (bristles) in the parapodia (Bauchot-Boutin & Bobin, 1954; Schroeder, 1967 in Fischer, 1999) and in some groups of new segmental muscle (Defretin, 1949 in Fischer, 1999).
Along with differentiation, other tissue degenerates. For example, in the male *Platynereis dumerilii*, a well studied nereidid that can be held and bred well in laboratory conditions, histolysis was found to affect about 80% of the irular muscle cells (a major structural element of the body wall) and 33% of the dorsal longitudinal muscle cells (Leisterer & Fischer, unpubl, in Fischer, 1999).
Fisher (1999) also reported transdifferentiation for the retina (light sensitive membrane) of nereidids in 1963. Defretin (1949) and Wissocq (1977) described transdifferentiation for the large longitudinal and for several segmental muscles. Later, Fisher (1999) found that after transdifferentiation these muscles resemble other fast-contracting tubular muscle cells and gained a function in propelling the nereidids when swimming. Wissocq (1970 in Fischer, 1999) found this phenomenon again in syllids (a family of polychaetes). The process of transdifferentiation of atokous muscle cells as opposed to a differentiation from previously undifferentiated cells has been concluded from the absence of undifferentiated cells and of mitoses before and during transformation (Fisher, 1999)

Another example of tissue differentiation and transformation in polychaetes is the *Neiris virens,* another well studied polychaete in process of epitoky. This polychaete is found in shallow marine soft-bottoms of the Northern hemisphere (Bass and Brafield, 1972; Dean, 1978; Wissocq, 1978; Kristensen, 1984; Hoeger, 1991; Fischer and Hoeger, 1993; in Chatelain et al, 2007; Breton et al, 2003; Franke, 1999). In this species, sexually matured males leave their burrows and swarm before spawning. N. virens is strictly semelparous: it breeds only once during its lifetime.
Accompanied with the swimming phase a range of epitokous modifications occur: enlarged parapodia, formation of natatory chaetae (the bristles used for swimming), diminution of the gut, histolysis of the body wall and reorganisation of body musculature (Bass and Brafield, 1972; Wissocq, 1978; ; Fischer and Hoeger, 1993 in Chatelain et al, 2007; Hoeger, 1991) .

2. Physiological transformation

Along with the physical modifications, nereidid polychaetes undergo considerable changes in the metabolism: a growth of the vascular system and respiratory surface, suggesting a respiratory adaptation as a mean of improving the swimming ability of the worms (Clark, 1961 in Chatelain, 2007). The observation that epitokous *N. virens* show significantly higher O_2 consumption compared to atokous

worms is consistent with the hypothesis of increased respiratory capacity during the period of increased muscular activity when the worms are swarming (Schöttler, 1989)

3. Timing of epitokous transformation

Spawning events in the reproducing part of the population is often remarkably synchronised. Earlier reports by Markert et al. (1961) confirm this for the epigamous Odontosyllis species *Odontosyllis enopla* (polychaete of the syllidae family). The epitokes of this species appear for a short mating event at the sea surface along the Bermudian coast 57 ± 1 minute after astronomical sunset and in relation to a certain lunar phase (Fischer & Fischer, 1995 in Fischer, 1999). This precision of timing has also been witnessed in the polychaete *Palola viridis* by Hauenschild et al. (1968 in Fischer, 1999). The emergence of the epitokes is timed by a combination of annual, lunar and dial rhythms that occurs only once a year at a specific time at night (Caspers, 1984)
There are many examples of lunar periodicity swarming (Olive & Clark, 1978 in Fischer, 1999). The first case in which the existence and timing stimulus of an endogenous lunar rhythmicity has been proven experimentally is that of the *P. Dumerilii* Hauenschild in 1956 (Fischer, 1999). Later, in 1980, 1985 and again in 1986 Franke proved the same phenomenon in *Typosyllis prolifera*.

Variation in day length (photoperiod) can influence reproductive activity (e.g. *Typosyllis prolifera* and *Neanthes limnicola*), as well as daylenght regime in the oligochaete *Stylaria lacustris.* For the latter the daylight regime is important for the choice between sexual or asexual reproduction (Schierwater & Hauenschild, 1990).
The precise timing of spawning in epitokes requires a link between external signal parameters and a physiological mechanism controlling sexual maturation. Seawater temperature and salinity may act as a signal for breeding as well (Goerke, 1984 in Fischer, 1999; Frey & Leuckart, 1985 in Fong, 1991)

4. Why going pelagic?

Epitoky and rapid swimming may have evolved as a means to ensure the encounter of sexual partners and to scatter the eggs in the midwater, where larvae are hatching just in the right place for their meroplanktonic style of life (Fischer, 1999).
In the nereidid polychaete N. virens, only the sexually matured males leave their burrows and swarm before spawning. They release their gametes in the sea, while the females apparently spawn in their burrows. In this way the females minimize their predation and avoid adverse environmental conditions that the males encounter (Bartels-Hardege and Zeeck, 1990)

Semelparous versus iteroparous species
Epitoky is mostly considered a one way process which implies a drastic reconstruction of the body tissue and is accompanied by a massive consumption of resources that will result in death after reproduction. This is the case in semelparous species, but some polychaete species have developed a strategy for an iteroparous life, allowing the worms to survive reproduction which makes them able to reproduce more than once in a lifetime. There are two known strategies: schizogamy and epigamy.

The process of *schizogamy* is characterised by budding: instead of entirely transforming into an epitoke, the atokous polychaete buds off a separate epitoke. Only the posterior part of the polychaete transforms into epitoke and becomes pelagic for some hours or days (Fischer, 1999).
A great example of this phenomenon is that of the *Palola (Eunice) viridis*. For more than 50 years only the posterior ends of this polychaete were known, until their coral-inhabiting 'stocks' were found and identified as '*Eunice*', which is now known as *Palola*. Slow communication in the steamship era and heated discussions about nomenclatural priority made that even the pygidium (posterior body part) of the

swarming epitoke of the 'Atlantic palolo' *Eunice fucata* was described as the 'head' of the worm. This led erroneously to the description of a new genus (Ehlers, 1900 in Fischer, 1999).
There is yet another type of schizogamy. In the polychaete *Autolytus prolifer*, a chain of short-lived extremely rapidly swimming male stolons or a chain of female stolons is budded off. Once detached these buds become pelagic and after pairing, they form a brood pouch and brood their eggs for a couple of days while living in the plankton (Fischer, 1999).

Some syllids practice yet another way to become epitokous: metamorphosis to an epitoke is quite similar to that of other related polychaete families, but without the degeneration of the digestive system. Another difference is the way of releasing gametes into the ocean water. As nereidids release their gametes by 'exploding' (they rupture their body wall), some syllids use modified nephridia (glandular excretory organs). In this case the syllids are able to return to the ocean floor and partly revert back to their atokous form, while nereidids do not survive their epitokous stage. This form of epitoky is called *epigamy* (Malaquin, 1893 in Fischer, 1999).
Schizogamous polychaetes put only parts of their body at risk while epigamous epitokes, becoming pelagic as a whole, always risk predation during their short pelagic phase of life and therefore might risk the loss of their whole reproductive potential (Fischer, 1999).

RESEARCH

Article 1

Epitoky in Nereis (Neanthes) virens (Polychaeta: Nereididae): A story about sex and death.

Étienne Hébert Chatelain, Sophie Breton, Hélène Lemieux, Pierre U. Blier (2007)

Introduction

In 2007, Étienne Hébert Chatelain et al. did a comparative study on immature atokous worms and mature epitokous males and females of the species *Nereis virens*. Metabolic changes during epitoky are the main subject in this study, and by studying the metabolic pathways and enzymes involved in the different energy producing pathways Chatelain et al. looked at the differences between mature males, mature females and atokous worms.

Nereis virens

Little is known about biochemical changes that nereidids undergo when they become mature and enter epitoky. When bentic males mature and become pelagic swimmers, drastic changes need to happen in both muscle tissue and metabolics to cope with the high energy bursts during the few days they swarm and reproduce.
The normally anaerobe worms need to change their metabolic pathways into an aerobic state to accomplish fast swimming. *N. virens* do not survive reproduction. Redox stress could play a big part, as well as starvation. As the digestive capacity changes as well, there is no feeding during the swarming pelagic period and the worms have to count on their reserves.

The epitokous *N. virens* individuals for this research were collected in an intertidal sandflat in Ste-Luce, Québec, Canada, in May 2003 and 2006. Atokous individuals were collected in the same area in

September 2003 and 2006. The polychaetes were separated in three groups: epitokous males, epitokous females and atokous worms and the average mass per group was measured.
To research the metabolic changes epitokous males undergo, five metabolic pathways were measured for each separate group:

- **(I)** **Mitochondrial aerobic metabolism** : → the electron transport system, NADH dehydrogenase, cytochrome c, reductase, cytochrome c oxydase and citrate synthase.
- **(II)** **Anaerobic metabolism** → activities of pyruvate kinase and lactate dehydrogenase.
- **(III)** **Amino acid metabolism** → measured with aspartate aminotransferase.
- **(IV)** **Defense capacities against oxidative stress** → estimated by the activities of superoxide dismutase and catalase.
- **(V)** **The digestive capacity** → measure of trypsin and lipase activities

Results

(I) Mitochondrial aerobic metabolism

The average mass was not significantly different among epitokous females, epitokous males and atokous worms (P=0,8), whereas epitokous females had significantly higher protein content (P<0.01) than both epitokous males and atokous worms

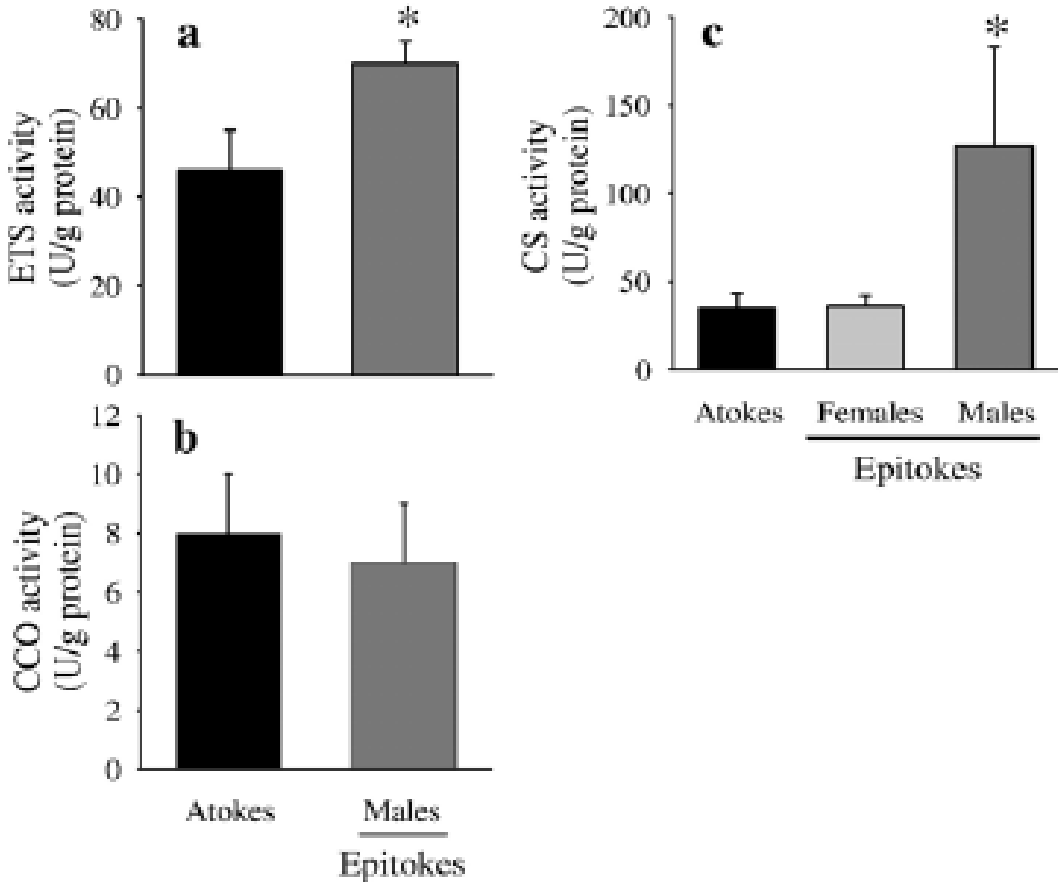


Fig. 1

Chatelain et al. found that the ETS activity (Electronic Transport System, accepts energy from carriers and stores it to a form that can be used to phosphorylate ADP) was significantly higher in epitokous males than in atokous worms (fig. 1a, $P<0.01$). There was no significant difference in **CCO** activity (cytochrome c oxydase) between these groups (fig. 1b, $P=0.41$). **CS** activity (Citrate Synthase, an enzyme that catalyzes the first step in the citric acid cycle and is commonly used as a quantitative enzyme marker for the presence of intact mitochondria) was significantly higher in epitokous males than in both atokous worms and epitokous females (Fig. 1c, $P<0.01$)

(II) Anaerobic metabolism

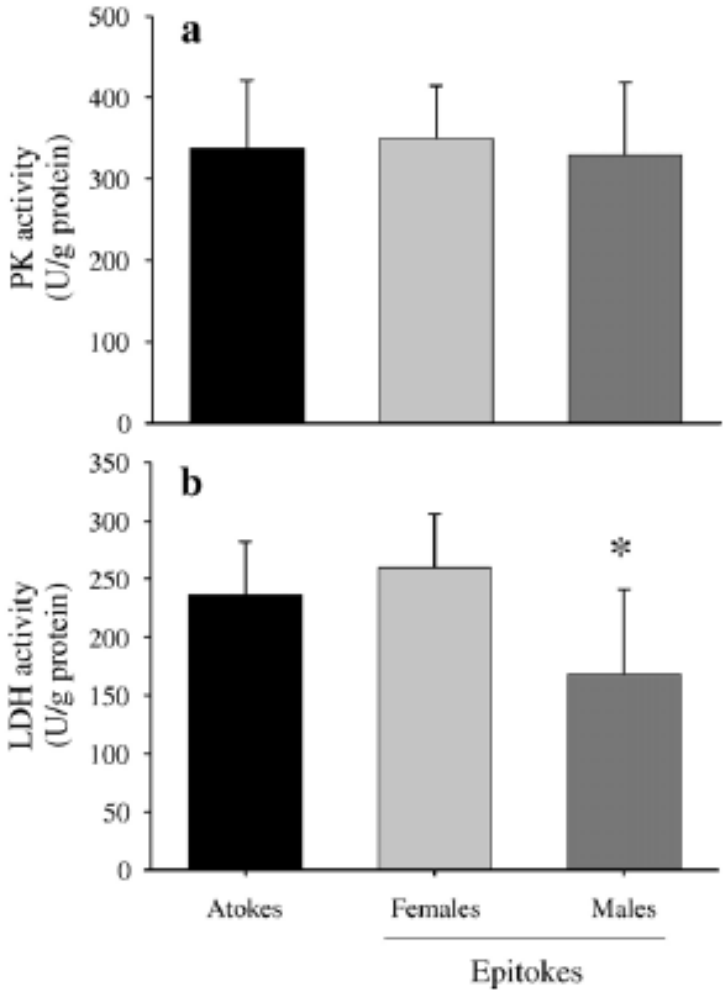


Fig. 2

There was no significant difference for **PK** activity (Pyruvate Kinase, an enzyme used by red blood cells that helps convert glucose to energy when oxygen is low) among the three groups (Fig 2a, $P = 0.29$). **LDH** activity (lactate dehydrogenase, an enzyme that catalyses the reversible interconversion of pyruvate and lactate) was significantly lower in epitokous males than in both atokes and epitokous females (Fig 2b, $P<0.01$), which suggests a decreased anaerobic metabolism in epitokous males.

(III) Amino acid metabolism

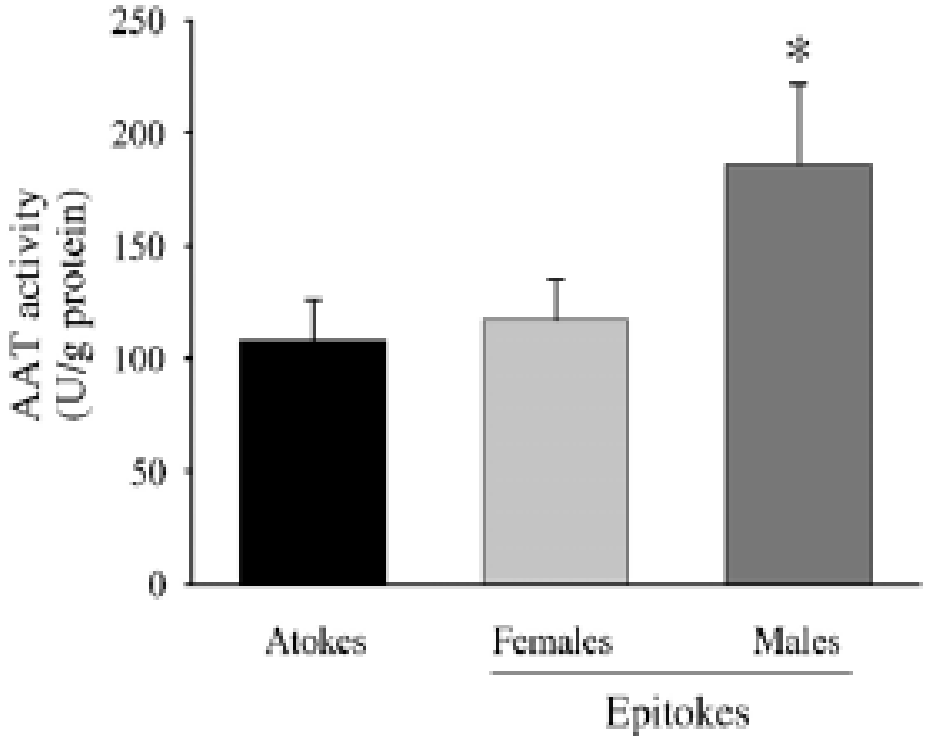


Fig. 3

Figure 3 shows a significant higher **AAT** activity **(**aspartate aminotransferase, an enzyme involved in the transamination of amino acids and leaks from tissues as a result of cellular damage), suggesting epitokous swarming males suffer from greater cell damage than the non-swarming epitokous females and atokous worms.

(IV) Defense capacities against oxidative stress

There were no significant differences between atokes and epitokous males (P=0.33) for **SOD** activity (Superoxide Dismutase, a class of important antioxidant enzymes that catalyse the dismutation of superoxide into oxygen and hydrogen peroxide) as well as for **CAT** activity (Catalase, catalyses conversions of hydrogen peroxide to water and molecular oxygen, P=0.67) and **GPX** activity (Glutathione Peroxidase, an anti-oxidant enzyme, P=0.83)
These enzymes were not measured for epitokous females because there were no significant differences in enzyme activity between females and atokous worms.

(V) Digestive capacities

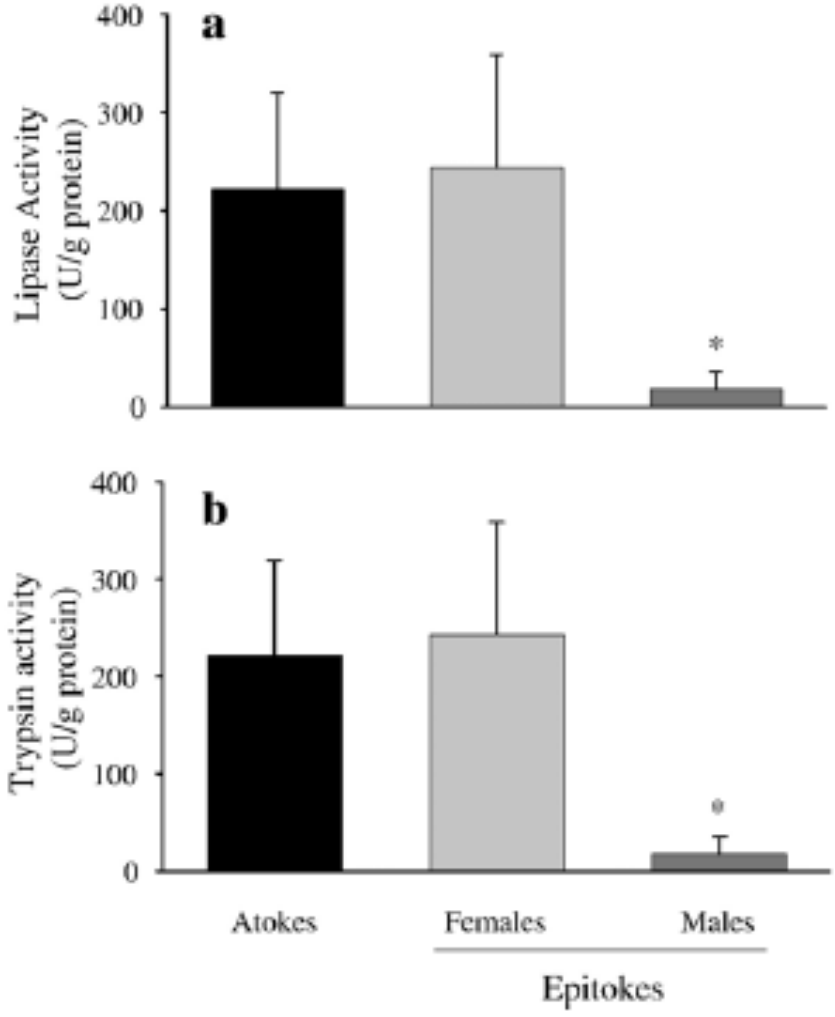


Fig. 4

Both Lipase (fig. 4a) activity and Trypsin (fig. 4b) activity were significantly lower in epitokous males than in epitokous females and atokous worms ($P < 0.01$), suggesting a decrease in digestive capacities.

Discussion

The results show that epitokous males of the species N. virens undergo drastic metabolic modifications to afford swimming and spawning.

Switch to aerobes

The increase of CS and ETS activities and the decrease of LDH, indicates that epitokous swimming males have a higher aerobic activity and a lower anaerobic capacity. All three of these changes in mitochondrial aerobic metabolism show that the body musculature has changed in order to provide enough energy for sustained swimming.
In a comparative research done by Schöttler in 1989, a 3-fold increase in VO_2 max (maximum amount of oxygen per minute) and glutamate dehydrogenase (a mitochondrial enzyme) and a 7-fold decrease in lactate dehydrogenase in N. Virens was found. These findings give prove that the size and number of mitochondria in epitokous worms increase during epitoky and that the males are adapted to aerobic activity.
Becoming aerobic is essential to enable epitokous males to swim before they release their gametes, but there is another problem that is solved by becoming aerobic. The major end product of anaerobiosis is lactate, which is slightly toxic. If the epitokous males would stay anaerobes during their swarming period, they would likely be toxicated and will not be able to swim for a long time. By becoming aerobic, the worms will have more energy to swim, the toxication problem will be solved and thereby their reproductive success enlarged.

The fact that there was no significant difference between the atokous worms and the epitokous males for CCO activity is unexpected. CCO, the terminal enzyme of the mitochondrial respiratory chain (Chatelain et al. 2007), is expected to have higher activity in the epitokous males, as transforming into aerobes also means higher oxidative stress. The found results might suggest that an increase in CCO activity is not required to improve the aerobic quality.
When ETS activity increases, the reduction state of the entire respiratory chain is increased. This could lead to increased production of ROS, reactive oxygen species. ROS are very small molecules that can damage other molecules and cell structures of which they are part. In absence of CCO responsiveness, this chain reaction will generate oxygen damage (Chen et al., 2003 in Chatelain et al., 2007).

There is an alternative route for electrons in times of oxidative stress: the alternative terminal oxidase (AOX). Through this pathway, respiration can be maintained when the cytochrome pathway is poorly functioning. However, this alternative route cannot match to the original CCO pathway. AOX bypasses several proton-pumping steps, which will lead to a reduce in ATP generation (McDonald and Vanlerberghe, 2004 in Chatelain et al., 2007).

Epitokous males show a higher AAT activity than epitokous females and atokous worms. This enzyme gives away the presence of cellular damage, and may give evidence that both reorganisation of the body musculature and histolysis of the body wall are taken place.

Overall, a higher mitochondrial metabolism was observed for the epitokous males. This could lead to an overall reduction of the mitochondrial respiratory system, which leads to an increase of ROS production. ROS is capable of damaging DNA, proteins and lipids and can therefore accelerate aging (Beckman and Ames, 1998; Barja and Herrero, 2000; Das et al., 2001; Bokov et al., 2004 in Chatelain et al., 2007).
The effect of oxidative damage can be questioned. As both epitokous males and females die within 3 days after swarming, and the females do not leave their burrow and thereby do not cope with oxidative stress, does oxidative damage have a significant impact on long term survival? Not finding a significant increase in superoxide dismutase, catalase and glutathione peroxidase (antioxidant enzymes) between epitokous males and atokous worms even though epitokous males show an increase in aerobic capacity support this

questioning. Future work is needed to determine the importance of oxidative damage during the short reproductive state of both epitokous males and females.

Digestive system transformation

Food deprivation may also play a great role in the rapid natural death after spawning. The results show significantly reduced digestive capacities in epitokous males compared to both atokous worms and epitokous females, but no significant difference in digestive capacities and AAT were found in epitokous females compared to atokous worms. As epitokous females do not swarm, they are able to continue feeding while maturing their eggs in the safety of their burrows. This feeding behaviour during egg maturation has already been observed for the female *Hediste diversicolor* (Clark, 1961)
Therefore food deprivation might have a great influence on the death of swarming epitokous males, but not on the epitokous females. Epitokous females do not die of starvation, but through ruptures in the body wall when they are spawning (Hofmann, 1964)

Conclusion

This article provides a clear view on changes in both metabolic patterns and physical changes occurring in epitokous males of the polychaete N. Virens. Considering what changes might occur when epitokous males are prepared for a sudden swarming lifestyle, I my opinion Chatelain et al. did well in combining both metabolics and digestive systems and comparing the different compounds between epitokous males, epitokous females and atokous worms. I think it might have been interesting to separate the atokous worms in both males and females as well to see if there already is a difference in metabolics, although I could not find any prove that it is possible to separate the sexes in the atokous stadium.

The results are quite clear: the differences found between swarming epitokous males and non-swarming atokous worms and epitokous females suggest an adoption for a short pelagic lifestyle. Starvation seems to be the main reason why epitokous males die within three days after spawning, as swarming demands lots of energy and they do not feed during their swarming period. Redox stress might also play a part, as the higher aerobic capacity is not correlated with an increase in antioxidant enzymes activities.
Chatelain et al. suggested that further research on mitochondrial metabolism, ROS production and oxidative damage is needed to better understand the part of oxidative stress as a cause of death in the swarming epitokous males of the N. virens.
I think that a comparative research with epitokous males of an epigamous species to compare oxidative stress and ROS production in both species could be interesting, as epigamous matured males also swarm, but do not die after reproduction. If these worms do show significant differences in anti-oxidant capacities compared to epitokous males of the species *N. virens*, it will become more certain that oxidative stress plays a part. If for epigamous species the other differences (metabolic and digestive) between matured males and females are comparable with those of the epitokous males and females of *N. virens*, oxidative stress might indeed play a big part in death after reproduction. However, it is known that the difference between epigamous syllids and related epitokous species lies in the degeneration of the digestive system, which suggests that food deprivation makes a difference between dying and surviving reproduction. Epitokous males of N. virens might therefore simply starve after reproducing, knowing that they do not feed during swarming and use up all their resources doing so. Still, oxidative damage might be as important, and therefore I think such a comparative research could give some more answers.

The last issue I would like to point out is that Chatelain et al. did not measure ETS and OCC in epitokous females but did not explain why this was unnecessary. ETS accepts energy from carriers and stores it to a form that can be used to phosphorylate ADP, and in my opinion this is important for high energy requirement and therefore there has to be a difference in epitokous males and females as the males need much energy for swarming.
Overall, this article provides a great basis for further research, as not much research has been done yet on the subject.

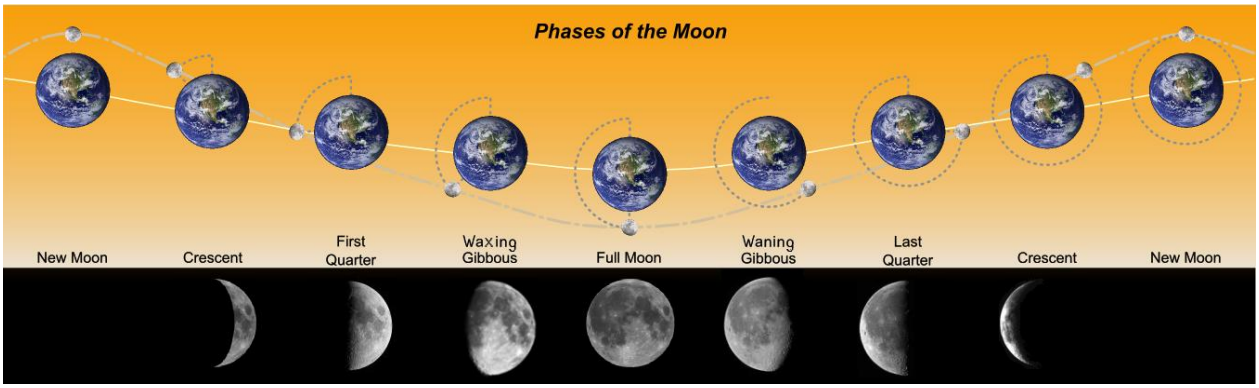
Article 2

Lunar control of epitokal swarming in the polychaete *Platynereis bicanaliculata* (Baird) from central california

Peter P. Fong (1993)

Introduction

A lot of research has been done on polychaete worms when it comes to lunar control of reproductive events in marine animals. In 1955 Hauenschild did an experiment on a Mediterranean population of the polychaete *Platynereis dumerilii* using artificial moonlight. He tested whether moonlight could synchronize the timing of epitokal metamorphosis and swarming, and found that the worms mainly swarmed during the period of new moon.
Worms have been observed swarming in Monterey Bay during the spring and summer on moonless nights when extreme high tides occur at midnight (Ricketts et al., 1985 in Fong, 1993).



In this study, Peter P. Fong practiced laboratory experiments using artificial moonlight to test the effect of lunar cues in the control of the timing of epitokal swarming in a Monterey Bay population of *Platynereis bicanaliculata*. *P. Bicanaliculata* is a heteronereid species, which means that both sexually matured adults swarm.

The polychaetes of the species were collected in 1989, from surfgrass-algal mats on an intertidal mudstone reef at Soquel Point, (36°57'N; I 22"O'W). The worms in the mats were transported to the laboratory, removed from their tubes and counted and placed in tubs (150 worms per tub) covered by light-tight wooden boxes and supplied with running sea water. They were given algae and surfgrasses to rebuild their tubes. Each tub was illuminated by a fluerecent light to simulate daylight. Daylights were controlled by time switches. To simulate moonlight, a dim incandescent night light was affixed into each wooden box.

Fong placed a stand pipe in each tub which he covered with a plastic mesh to catch the sexually mature worms when they started swarming. The worms adhering to the mesh were counted each day. The experiments were run until all worms had swarmed.

Platynereis bicanaliculata

Two separate experiments were carried out. In both experiments the amount of artificial moonlight was changed while all other conditions were kept the same.

Experiment I: Testing the effect of artificial moonlight on the timing of swarming
For this experiment, 450 worms were collected on 9 April 1989, and placed in three tubs (150 worms/tub). Artificial moonlight was on for either 6 or 7 nights (12 hours a night):

- Tub 1: amount of artificial moonlight centered around the period of ambient full moon
- Tub 2: amount of artificial moonlight centered around the period of ambient new moon
- Tub 3: constant artificial moonlight (control)

All three treatments were maintained under in-phase daylength regimes.

Experiment II: Ascertain what part of the lunar cycle *Platynereis bicanaliculata* uses as timing cues for swarming
All worms were collected at 12 November 1989. Clumps containing worms were weighed and equal amounts placed in each of the three tubs with artificial moonlight for 14 nights (12 hours a night):

- Tub 1: amount of artificial moonlight centered during the period of ambient full moon
- Tub 2: amount of artificial moonlight centered during the period of ambient new moon
- Tub 3: constant moonlight (control).

These two experiments are comparable. If the worms swarm on the beginning of the moonlight cycle, then a swarming pattern similar to that of experiment 1 is to be expected. If the end of the moonlight cycle is the cue, swarming should occur only after the 14 nights of artificial moonlight have ended.

Results

Experiment 1: the effect of artificial moonlight for 6-7 uninterrupted nights per month:
The figure below shows the running averages of swarmers for the three conditions:

- Artificial moonlight for 6-7 uninterrupted nights centered around the period of ambient full moon (A)
- Artificial moonlight for 6-7 uninterrupted nights centered around the period of ambient new moon (B)
- Continuous artificial moonlight as a control experiment (C).

The close circles indicate ambient new moon, and the open circles ambient full moon.
Open bars indicate times of artificial moonlight. The closed bars indicate times without moonlight Artificial

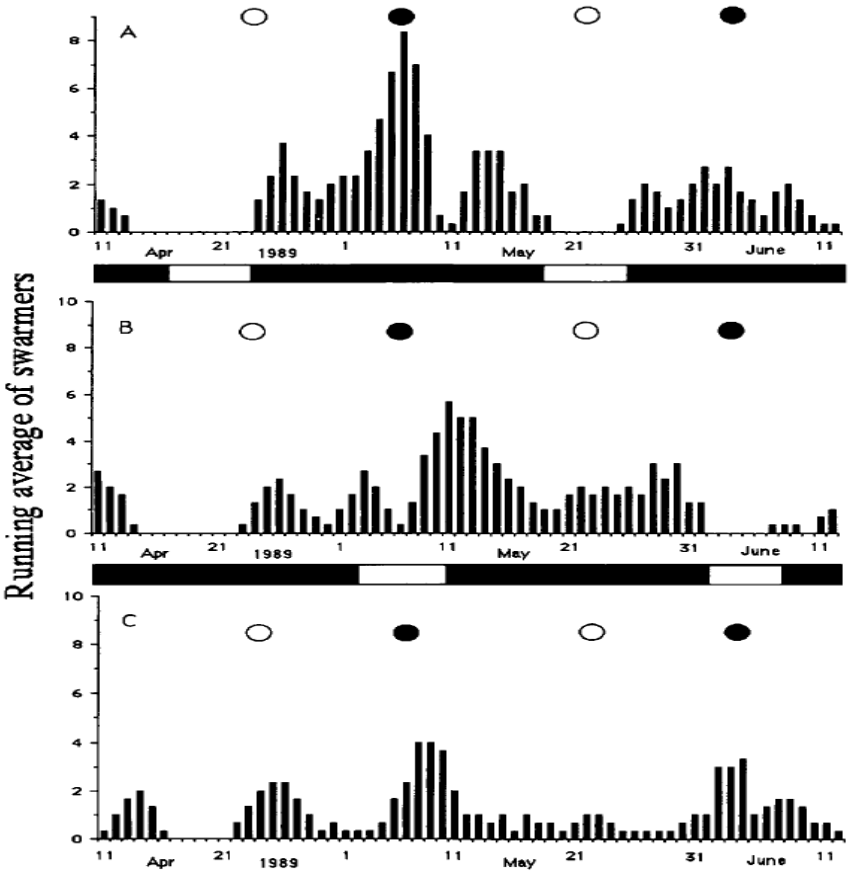


Fig. 5

Fig. 5A shows that moonlight had a strong effect on the timing and frequency of epitokal swarming. The swarming began immediately after the artificial moonlight was turned off and ceased when the moonlight was turned on again.
Fig. 5B shows a different pattern, but at the beginning of the experiment it seems that the worms are somehow triggered to swarm during "real" ambient new moon, even though the artificial moonlight was not turned on until several days later (there was no experimental cue for swarming). In the second cycle the worms do not swarm on nights with artificial moonlight.
The control experiment (fig. 5C) shows almost the same swarming pattern at the beginning of the experiment and shows peaks centered around ambient new moon. For both experiments in phase with ambient new moon (B) and the control experiment (C) the worms start swarming somehow randomly as the experiment lasts.

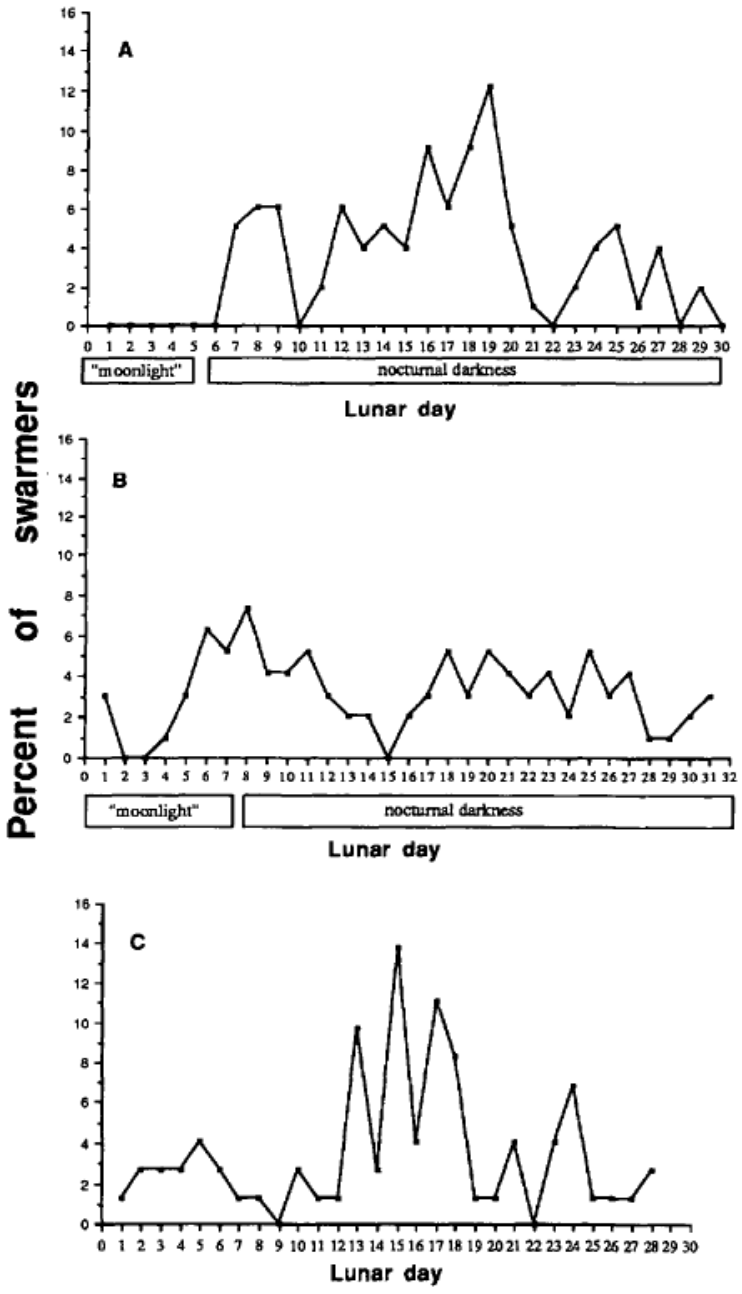


Fig. 6

Figure 6 shows the percent of swarmers for the three experiments (A, B, C). The worms in phase with ambient full moon (fig 6A) started swarming after the artificial moonlight was turned off. Peak swarmings occurred between the 16th and 19th lunar day. The worms in phase with ambient new moon showed a more random pattern (fig. 6B). Swarming began on the first lunar day, even when the artificial moonlight was on. Peaks occurred between the 6th and 8th lunar day.

The worms in the control experiment swarm constantly (fig. 6C), but showed peaks on the 13th, 15th and 17th lunar day. This pattern somehow resembles the pattern shown in fig. 6A (peak swarming dates are close to each other). Considering fig 6B, the worms seem to swarm more randomly, not showing definite peaks as are shown in fig. 6A and C.

Experiment 2: artificial moonlight for 14 uninterrupted nights.

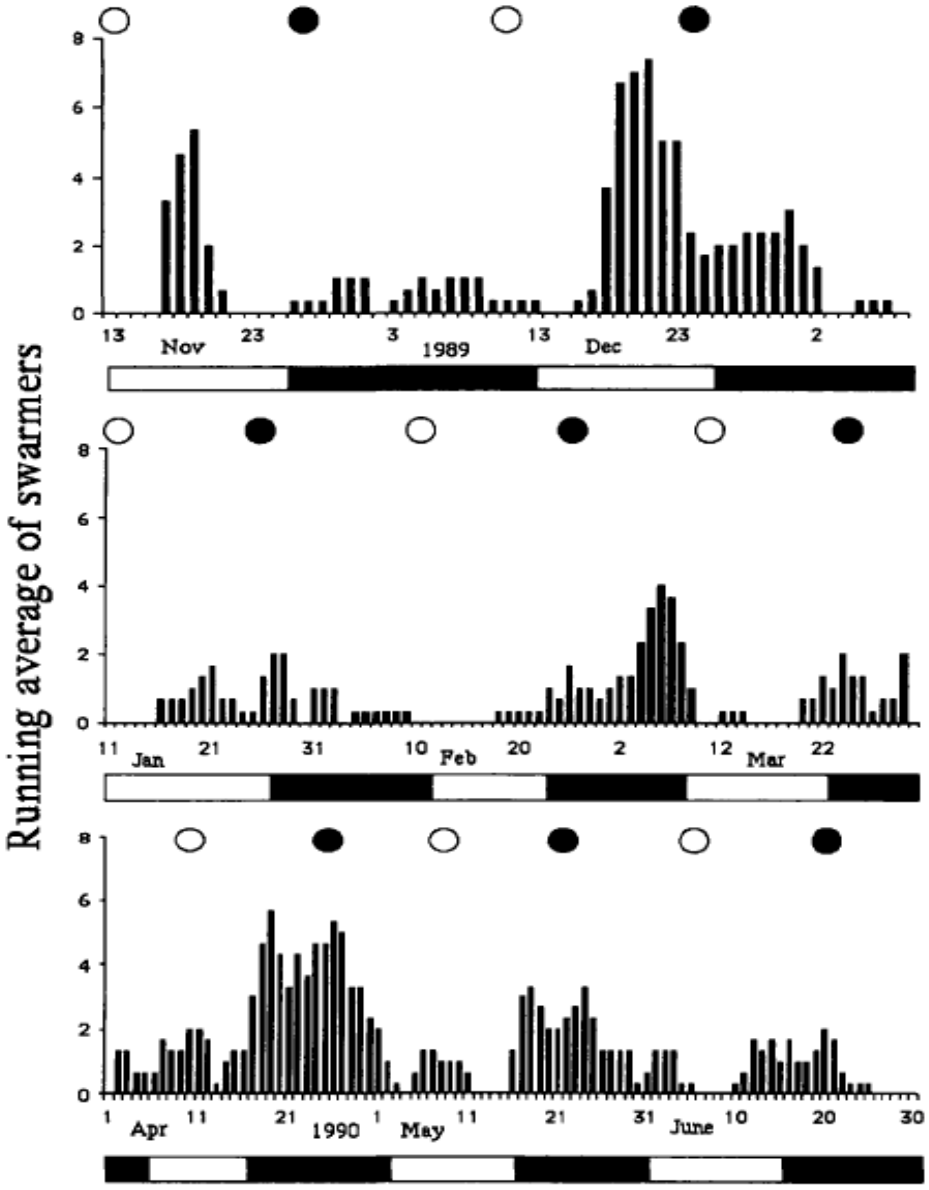


Fig. 7

Fig. 7 shows the running averages (calculated the same way as in fig. 5) of swarmers exposed to artificial moonlight for 14 uninterrupted nights during the period of ambient full moon.
Worms swarmed both on "moonless" and "moonlit" nights. In the beginning of the experiment the worms show field entrainment and reacted to their endogenous rhythm. From January till March the worms swarmed randomly and do not seem to react to the artificial moonlight. From February till June most worms started swarming on nights without artificial moonlight, but still few worms swarmed on "moonlit" nights. The irregularity shown on the months before could be explained by the worms re-setting an internal clock to a different lunar cycle.

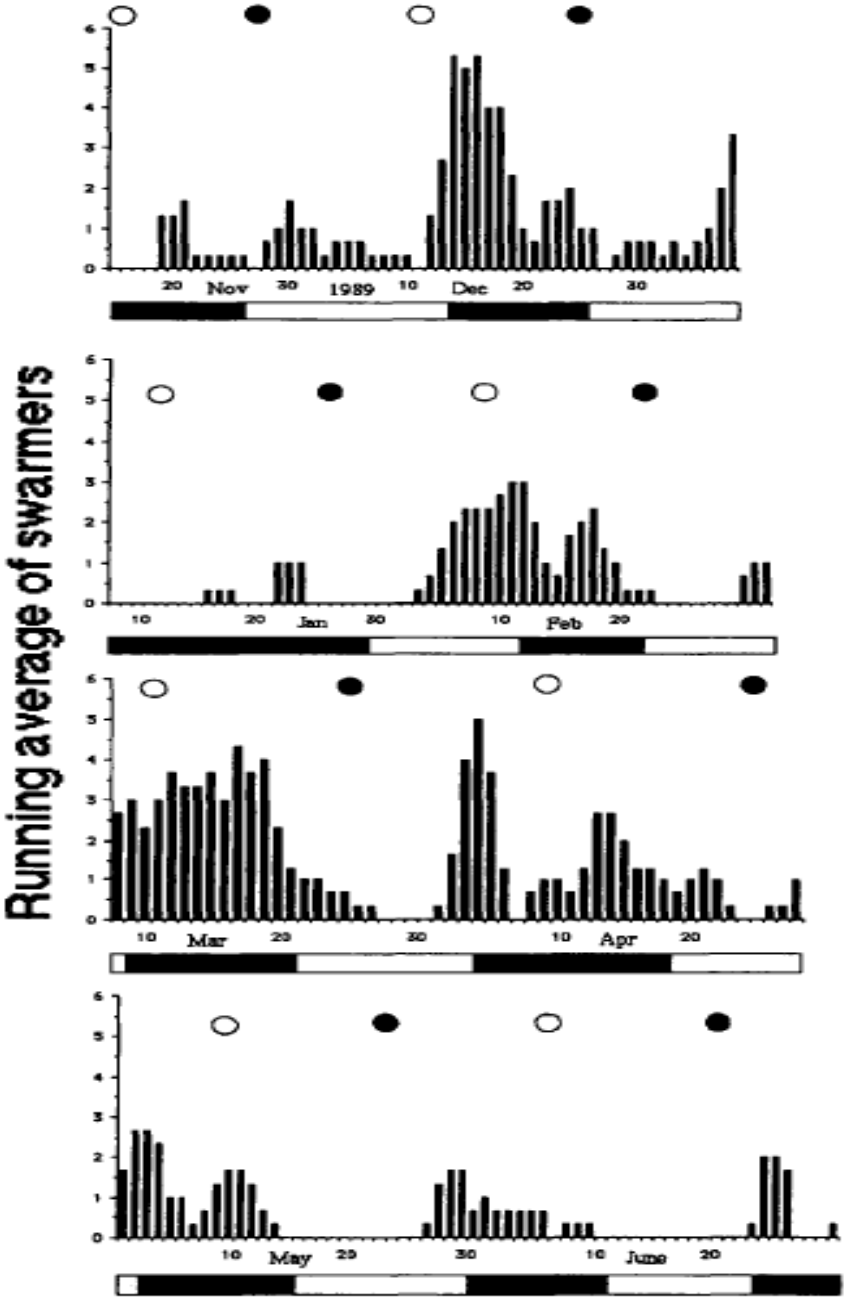


Fig. 8

Fig. 8 shows the running averages of swarmers exposed to artificial moonlight for 14 uninterrupted nights during the period of ambient new moon from November 1989-June 1990. From November until December the worms show an irregular swarming pattern, but there are definite peaks centered around ambient full moon (after the first 14 days of artificial moonlight). From January until end February little swarmings occur, but most swarmings seem to happen around ambient full moon. In March and April again most swarmings seem to occur around the period of ambient full moon. In May and June almost no worms swarmed on nights with artificial moonlight. Swarming occurred mostly around ambient full moon.

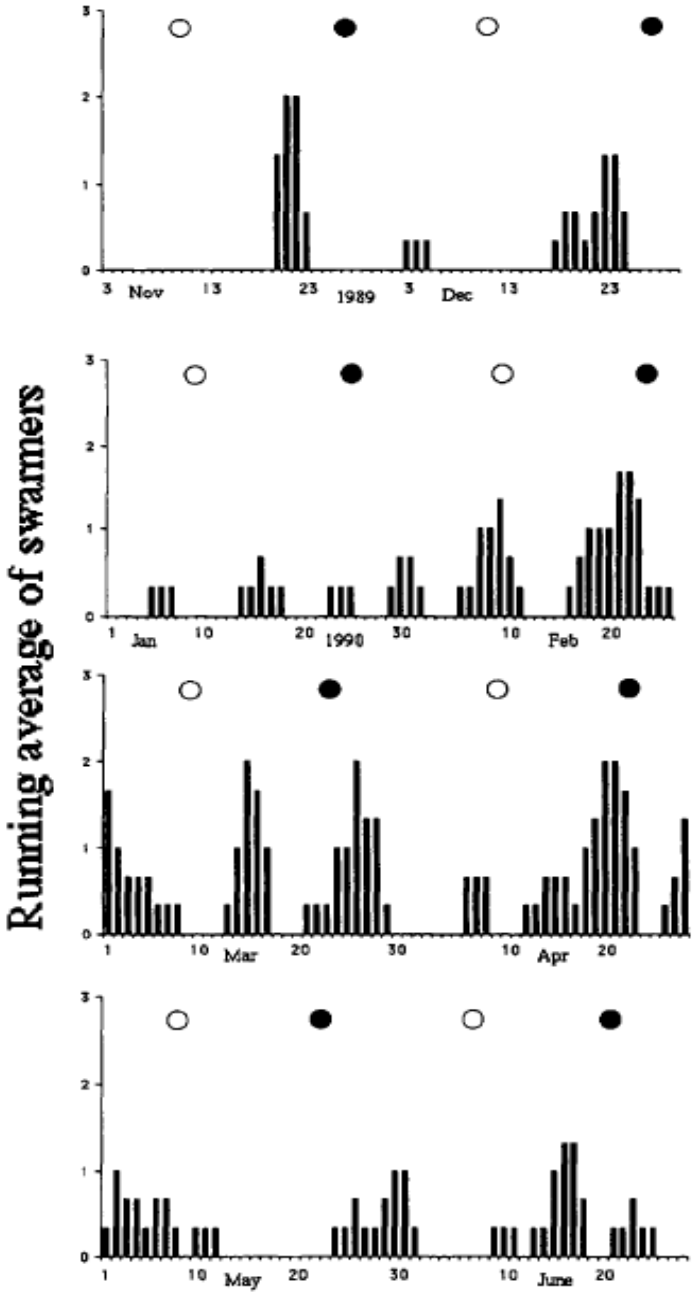


Fig. 9

Fig. 9 shows the running average of swarmers in constant artificial moonlight from November 1989-June 1990. The pattern shown here is significantly non-random. In November and December most worms swarmed at the end of ambient full moon, but in the following months the figure shows that swarming does not seem to synchronise with ambient full or new moon and becomes random. Again, in the months of January and February, little swarming activity has taken place. End February the average of swarmers is peaking slightly and continues in March and April, but none of the peaks show a clear pattern. In May and June the swarming decreases without showing a pattern to ambient new or full moon.

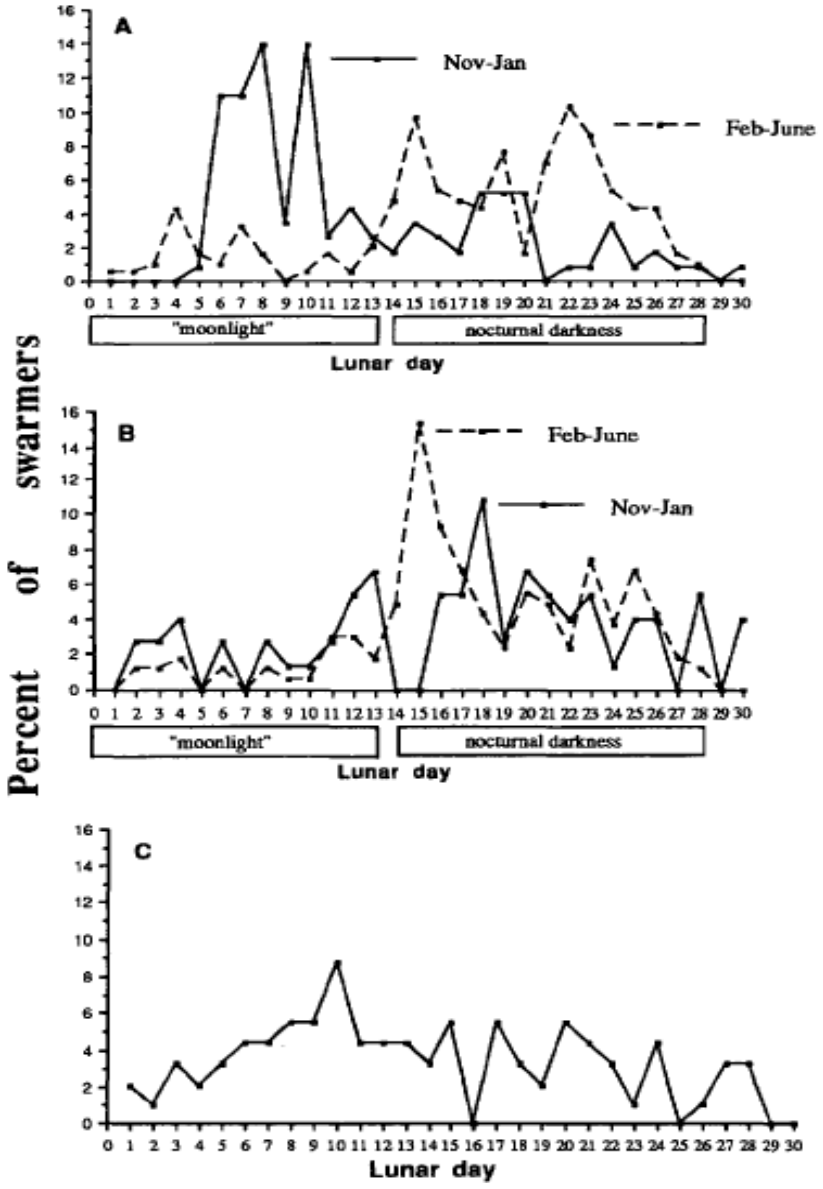


Fig. 10

Fig. 10 shows the percent of swarmers during lunar cycles (days after the first night of artificial moonlight) with A: artificial moonlight for 14 uninterrupted nights during the period of ambient full moon, B: artificial moonlight for 14 uninterrupted nights during the period of ambient new moon and C: constant moonlight.

The highest percent of swarmers during ambient full moon in November and December occurred between the 5th and the 10th day (fig. 10A), while artificial moonlight was on. In February-June the peaks were triggered to the right, and most worms started swarming after the artificial moonlight was turned off. The highest peak of swarmers occurred between the 21th and 23th lunar day. In fig. 10 B, where the worms were exposed to artificial moonlight during ambient new moon, most worms swarmed after the artificial moonlight was turned off. From February-June the figure shows a peak between the 14th and 16th lunar day. In November-January, the highest percentage of worms swarmed between the 17th and 19th lunar day. The worms exposed to constant artificial moonlight show no synchronised pattern. A little peak seems to occur between the 9th and 11th lunar day.

Discussion

In the first experiment, *Platynereis bicanliculata* exposed to artificial moonlight in phase with ambient full moon only swarmed on moonless nights. Moonlight might have a strong effect on epitokal swarming, but this swarming pattern could also occur because the worms were field entrained (as the experiment started immediately after the worms had been caught). This field entrainment has been demonstrated before by Franke in 1985 in the polychaete *Typosyllis prolifera* and it can be suggested that *P. bicanliculata* has the same kind of internal clock in reacting to moonlight.
Knowing this, I think more accurate results can be gathered when one does not start the experiment immediately, but first give the polychaetes some time to reset. One way this could be done is by dividing the animals in two groups, exposing one group to constant moonlight for about a month, and leave the other group in darkness. When the experiment is started, the worms will not be "set" as they were not given any cues during their incubation time. Hauenschild found a comparable solution: before he started his experiment in 1960, he exposed his immature worms to several periodical changes of the daily illumination time. In this way, the swarming of the polychaetes could not be influenced by an external time-giver.
Another solution might be starting the experiment earlier. This will solve problems as seen in experiment 1A, where it is not certain if the swarming pattern is because of field entrainment or if the worms truly respond to artificial moonlight. In my opinion, if Fong had started experiment 1 in November as well, he would have gotten clearer results because filtering out field entrainment would have played a smaller role in the experiment.

Considering experiment 1B, where the worms were exposed to artificial moonlight in phase with the ambient new moon, there is a comparative pattern with 1A: in the beginning of the experiment the worms seem field entrained as well, but in the second moon cycle the worms only swarmed on nights without artificial moonlight,. It seems that worms can be lab entrained to swarm on cycles of artificial moonlight lasting 6-7 nights, but it takes about a month for the worms to "reset". The field entrainment also exists in the control experiment with constant artificial moonlight. During the first cycle, the worms follow almost the same pattern as the worms in in phase with ambient full and new moon, but show no clear cycle pattern afterwards as constant moonlight does not seem to provide a clear cue for swarming.

In experiment 2, worms showed field entrainment in November and December, but very few swarmed from January to early February. Fong suggested that worms were "adjusting" or "re-setting" an internal clock to a cycle of 14 "moonlit" nights. The periodicity of such a clock is unknown but it might normally be set by the monthly cycle of moonlight. Those worms in experiment 2 waited 14 nights with artificial moonlight before swarming suggests that *P. bicanaliculata* uses the end rather than the beginning of the moonlight period as a cue to synchronize swarming.
Fongs observed that the polychaetes in his second experiment showed field entrainment in November and December, but very few swarmed from January to early February (the months after field entrainment would have ceased and lab-entrainment could take place). He suggested, explaining this phenomenon, which the worms were adjusting or re-setting in internal clock. According to field observations of Ricketts et al. in 1985, polychaetes of this species in the Monterey Bay swarm during the spring and summer. The fact that Fong saw just little swarming activity in January to early February could just be because the worms were not ready to leave their burrows as they had not completed epitokous metamorphosis. This of course could be linked to sea water temperature, but the sea water temperature in the lab could not have been very different from the sea water outside, as running sea water was used. Fongs suggestion about re-setting might be the explanation for few swarmers in January to early February, but I think that most worms were still atokous at that time.

Initially, the first night artificial moonlight was turned either on or off was within 1-2 nights of the ambient moon (either full or new). However, from November 1989 through March 1990, there were either 15 or 16 nights between ambient full and new moon, but laboratory moonlight changed only every 14

nights, Moreover, in late January 1990, the "moonlight-on" and "moonlight-off" was accidentally delayed by a few days. Thus, by April 1990, the timing of the "moonlight-on" or ″-off′ was near the center of each ambient moonlight period. This error might have influenced the experiment.

This study is comparative to Hauenschilds study of the swarming patterns of *P. dumerilii* from the Mediterranean Sea in 1960. Hauenschild found that the cessation of moonlight (beginning of ambient new moon) was the cue for completion of sexual maturation, onset of epitokal metamorphosis and swarming in this species. In his experiments he found that *P. dumerilii* starts swarming about 18 days after the artificial moonlight was turned off and proposed that this entrained swarming behaviour was caused by an underlying endogenous rhythm. This swarming synchrony was maintained for 4 months under constant conditions without artificial moonlight, after the artificial moonlight was turned off, which gives evidence for the existence of an underlying endogenous rhythm
Comparing the results found in this study with the results from Hauenschilds study of the *P. dumerilii* one can conclude that *P. bicanaliculata* from the Monterey Bay in California has an endogenous, moonlight entrained rhythm. Like *P. dumerilii, P. bicanaliculata* probably uses the decline from full moon to last quarter moon as a cue for swarming. Field observation of this species shows that the cue begins around 7 days after full moon and the worms show a reaction time of about 7 days as well (which explains the high frequency of swarmers around the time of new moon)

The difference between the lab experiments and field observations is that there is no gradual decline of moonlight in the lab. Here, artificial moonlight was either turned on or off, causing a shorter cueing period of about 1 day. This might explain the high percentage of swarmers on or near the 15^{th} lunar day in experiment 1 and near the 22^{nd} lunar day in experiment 2.
Fong suggestion that there exists a possible semi-lunar component to the overt swarming rhythm to explain the small swarming peaks around the period of ambient full moon in the first month of experiment 1, together with the peak swarming intervals of about 13 days in the "constant moonlight" treatments of both experiments 1 and 2. He suggested that this rhythm cannot be considered truly free-running since the daylength regimes (in phase with ambient) were not "constant". These free-running rhythms have been recorded before in *Platynereis dumerilli* from the west coast of France (Fage and Legendre, 1927; Durchon, 1970 in Fong, 1993), and the Adriatic Sea (Georgevitch, 1938 in Fong, 1993). I think that it can also not be called free-running because of the differences between the two experiments. In the first experiment the worms could still have been field entrained, while in experiment 2 the worms were caught earlier and became lab-entrained during the experiment. The small swarming peaks around the period of ambient full moon in the first month of experiment 1 might as well have been the result of field entrainment instead of a semi-lunar component, because the lights in the experiment were either turned "on" or "off" and not "dimmed". The peaks in the control situation (constant artificial moonlight) in both experiments 1 and 2 could show field entrainment. As a lunar cycle had a duration of ± 29 days, and the end of full moon is a trigger to swarm, it is to be expected that the worms swarm ± 14 days later if they are field entrained. Why the worms keep showing these swarming peak intervals of about 13 days even after experiencing "constant moonlight" for a few months, I do not know. As was shown from experiment 2, the worms used rather the end of the moonlit period as a cue to start swarming, you would expect no swarming at all after a few months, because there is no cue if the light is not turned off. Perhaps this phenomenon could explain some free-running semi-lunar rhythm, but I don't think that this is the case for the small swarming peaks around the ambient full moon in the first experiment.

References

- **Epitoky in Nereis (Neanthes) virens (Polychaeta: Nereididae): A story about sex and death**
  Étienne Hébert Chatelain, Sophie Breton, Hélène Lemieux, Pierre U. Blier, 2007

- **Reproductive and developmental phenomena in annelids: a source of exemplary research problems**
  Albrecht Fischer, 1999

- **Reproduction of the Syllidae (Annelida: Polychaeta)**
  Heinz-Dieter Franke, 1999

- **Lunar control of epitokal swarming in the polychaete Platynereis bicanaliculata (baird) from central California**
  Peter P. Fong, 2003

- **On a clocklike mechanism timing lunar-rhythmic reproduction in *Typosyllis prolifera* (Polychaeta)**
  Heinz-Dieter Franke, 1985

- **Lunar periodicity**
  C. Hauenschild, 1960

- **Phylogeny and evolution of reproductive modes in Autolytinae (Syllidae, Annelida)**
  Arne Nygren and Per Sundberg

- **Population structure of two northern hemisphere polychaetes, Neanthes virens and Hediste diversicolor (Nereididae), with different life-history traits**
  S. Breton, F. Dufresne, G. Desrosiers, P.U. Blier, 2003

- **Anaerobic metabolism in the lugworm Arenicola marina during low tide: the influence of developing reproductive cells**
  Udo Schöttler, 1989

- **Hydrolytic enzymes in the coelomic cells of the polychaete *Nereis virens* during sexual maturation**
  U. Hoeger, 1991

- **A Photoperiod Determined Life-Cycle in an Oligochaete Worm**
  Bernd Schierwater and Carl Hauenschild, 1990

- **Spawning periodicity and habitat of the palolo worm *Eunice viridis* (Polychaeta: Eunicidae) in the Samoan Islands**
  H. Caspers, 1984

- **Reproductive behaviour of *Nereis diversicolor* (Annelida: Polychaeta)**
  H.D. Bartels-Hardege and E. Zeeck, 1990

Websites

http://www.gpnotebook.co.uk
http://www.nlm.nih.gov
http://healthguide.howstuffworks.com/pyruvate-kinase-dictionary.htm
http://www.encyclopedia.com
http://www.catalase.com/cataext.htm
http://catalogue-of-organisms.blogspot.com
http://highered.mcgraw-hill.com/sites/dl/free/0072830565/198678/ch17.pdf