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Non-mammalian Hosts and Photobiomodulation: Do All Life-forms Respond to Light? †

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Abstract

Photobiomodulation (PBM), also known as low-level laser (light) therapy, was discovered over 50 years ago, but only recently has it been making progress towards wide acceptance. PBM originally used red and near-infrared (NIR) lasers, but now other wavelengths and non-coherent light emitting diodes (LEDs) are being explored. The almost complete lack of side-effects makes the conduction of controlled clinical trials relatively easy. Laboratory research has mainly concentrated on mammalian cells (normal or cancer) in culture, and small rodents (mice and rats) as models of different diseases. A sizeable body of work was carried out in the 1970s and 1980s in Russia looking at various bacterial and fungal cells. The present review will cover some of these studies and a recent number of papers that have applied PBM to so-called “model organisms”. These models include flies (*Drosophila*), worms (*C. elegans*), fish (zebrafish), and caterpillars (*Galleria*). Much knowledge about the genomics and proteomics, and many reagents for these organisms already exist. They are inexpensive to work with and have lower regulatory barriers compared to vertebrate animals. Other researchers have studied different models (snails, sea urchins, *Paramecium*, toads, frogs and chickens). Plants may respond to NIR light differently from visible light (photosynthesis and photomorphogenesis) but PBM in plants has not been much studied. Veterinarians routinely use PBM to treat non-mammalian patients. The conclusion is that red or NIR light does indeed have significant biological effects conserved over many different kingdoms, and perhaps it is true that “all life-forms respond to light”.

Graphical Abstract

Photobiomodulation (red or near infrared light) has been shown to have beneficial effects on many different life-forms. Although many pre-clinical studies have been carried out in mice or rats, and clinical studies in humans, it turns out that all life-forms from bacteria and fungi, to paramecia,

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Conflict of Interests

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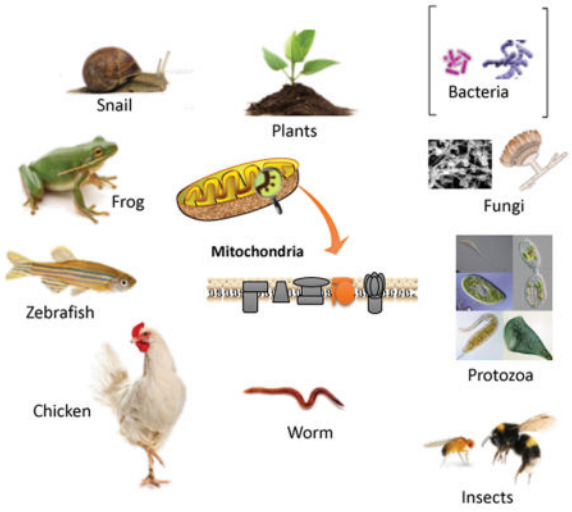
worms, insects, fish, amphibians, reptiles and birds respond. Even plants respond to NIR light differently to photosynthesis with visible light.



Keywords

Non-mammalian lifeforms; model organisms; photobiomodulation; low-level laser therapy; insects; worms and snails; aquatic creatures; birds

INTRODUCTION

Photobiomodulation (PBM) previously known as “low-level laser therapy” (LLLT) was discovered in 1967 by Endre Mester in Hungary. However, light therapy for a variety of diseases had been practiced for thousands of years first using sunlight, and then by electric lamps since their discovery in the 19th C by Davy (arc lamp) and Edison (incandescent bulb). Niels Finsen was awarded the Nobel Prize for physiology or medicine in 1904 for his work on treating cutaneous tuberculosis with blue light and smallpox with red light. In the 21st C it was realized that light-emitting diodes was equally as effective as lasers in most applications of PBM. The mechanisms of action of PBM have been intensively investigated starting with the work of Tina Karu in Russia (1). Karu was the first to attribute the effects of red and near-infrared light to photon absorption by the electron transport chain in mitochondria, and by unit IV cytochrome c oxidase (CCO) in particular (2). Since CCO has absorption peaks in the red (600–700nm) and near-infrared (NIR, 760–900 nm) regions of the spectrum (depending on its precise oxidation state), red and NIR light have been the most often employed wavelengths for PBM (3, 4). In recent years, it has become apparent that there are other chromophores operating in different regions of the spectrum (5). The most important alternative chromophore appears to be transient receptor potential (TRP) ion channels that can be activated by light or heat (6). An example is TRPV1 that can be activated by blue or green light (7, 8) and by longer wavelength NIR light (980 nm) (9) that targets water in the form of nanostructured exclusion zone water clusters (10). Regardless of whether CCO is activated by red/NIR light, or TRP ion channels are activated by blue/green/980 nm light, the cellular results have some aspects in common, but also display some

differences. Both pathways give rise to increased ATP and a brief burst of reactive oxygen species (5). However, the CCO pathway is more likely to lead to release of nitric oxide and the TRP pathway is more likely to give rise to changes in cytosolic and mitochondrial calcium levels.

The secondary effects of PBM can be short term in nature, such as increased blood flow, greater oxygen consumption, improved tissue ATP production etc. They can also be more long term in nature such as increased antioxidant defenses, activation of transcription factors and alteration of the expression pattern of cytokines and growth factors.

One pervasive observation in the field of PBM, is called the biphasic dose response curve otherwise known as the Arndt-Schulz law (11, 12). This states that as the dose of light (J/cm^2) is gradually increased, the beneficial response reaches a maximum at some point. When the dose is further increased beyond that maximum, the response declines until the baseline is reached again (13). If the dose of light is increased even more than that, a negative response starts to occur (inhibition) that might end up in cellular damage and even cell death.

Since many (if not most) of the mechanisms proposed to explain the cellular effects of PBMT are common to a lot of different life forms on earth, it has recently become possible to use a range of non-mammalian hosts, that have become popular in biomedical research laboratories throughout the world in recent years. These include worms, flies, fish, frogs and other “model organisms”.

MODEL ORGANISMS

The use of animal models has played a major role in the advancement of biomedicine and has been a cornerstone of medical progress for many centuries. Mammals such as mice, rats, rabbits and monkeys have traditionally been the preferred models for biomedical research due to their close evolutionary relationship with humans. However, the use of mammals in research has raised ethical and budgetary concerns, and has caused many laboratories and funding agencies to adopt the principle first elaborated by W. M. S. Russell and R. L. Burch as the “Three Rs”: Replacement, Reduction, and Refinement” (14). The Three Rs principle encourages the use of alternative model systems in addition to cell culture, which is already widely used in PBM research. These laboratory model systems include three important animal species, *Danio rerio* (zebrafish), *Drosophila melanogaster* (fruit fly) and *Caenorhabditis elegans* (nematode worm) that have now become widespread in biomedical research. These three model organisms have a rapid rate of reproduction with a relatively short generation time. They are easily grown in the laboratory (with no need of expensive animal housing) and do not require burdensome and time-consuming IACUC protocols. So far they have largely escaped the attention of animal rights protesters. Because they are living animals they are considerably more powerful than cell culture systems. In addition, thanks to the vast number of molecular and genetic tools available to study *Drosophila*, *C. elegans* and zebrafish, coupled with similarities in development and behavior, these organisms have come to serve as powerful alternatives to mammalian models to investigate genetics, diseases, and possible therapeutics.

Table 1 shows a comparison of the advantages and limitations of these three model organisms in comparison with the traditional laboratory mouse which is still the most widely used species in all of biomedical research.

C. elegans

C. elegans is a transparent, free-living, non-parasitic nematode. *C. elegans* larvae at hatching are 0.25 millimeters long and adults are 1 millimeter long with a maximum diameter of ~80 μm. This nematode can survive on a lawn of *Escherichia coli* bacteria growing on agar medium in Petri dishes, which makes it very easy and cost-effective to grow in the laboratory. *C. elegans* has a short life cycle (3.5 days to obtain fertile adults at 20°C) and adult hermaphrodites are highly prolific reproducers (>140 eggs per adult per day) (15). Males are rare in laboratory populations (1 out of 1000 individuals) and the hermaphrodites reproduce by self-fertilization, creating genetic clones of themselves.

The anatomical simplicity of *C. elegans* and lack of a central nervous system may be considered to be drawback in a model used to study mammalian biomedical responses. However, this simplicity has led to one of the key advantages: every single neuron has been identified and the connections between them (the connectome) are known (16). This is not the case for any other organism. In addition, with the aid of genetically encoded fluorescent proteins, processes such as axon growth, embryogenesis and metabolic processes can easily be studied in the living worm. Although the adult hermaphrodite has only 959 somatic cells and its entire nervous system contains a mere 302 neurons, *C. elegans* is still a sophisticated multicellular animal with different organs and tissues including muscle, skin, intestine, reproductive system, and glands.

Drosophila melanogaster

The fruit fly (*Drosophila melanogaster*) has been instrumental in important advances biomedicine (17). Many of the genes found to be critical for fly development were shown to also be important for human development. Drosophila is relatively inexpensive and easy to culture and there are very few ethical and regulatory restrictions on its use in the laboratory. The short life cycle (approximately 12 days to obtain a fertile adult fruit fly) and the large brood size (120 eggs per adult per day) (18)

The adult fly has organs that are functionally equivalent to the mammalian heart, lung, kidney, gut, and reproductive tract. The Drosophila genome was fully sequenced in 2000 and Reiter et al reported that 77% of 929 genes known to be involved in human diseases displayed a match within the fruit fly genome (19)

Danio rerio

The zebrafish (*Danio rerio*) is a small freshwater teleost fish widely used in developmental research. Zebrafish embryos are transparent and develop outside the mother, making it relatively easy to study different stages of development using optical imaging techniques. Zebrafish genome contains numerous duplicate genes (20) and this feature often hinders the generation of knockout strains, as approaches that disrupt one gene copy likely will not disrupt the second copy. The zebrafish genome has been fully sequenced and its annotation

is an ongoing project. A recent study indicated that 84% of the genes known to be associated with human disease have a counterpart in the zebrafish genome (21).

Galleria melonella

Larvae of the greater wax moth *Galleria mellonella* have recently been used as model hosts for studying pathogenic microorganisms as an alternative to vertebrate animals (22). The model has been used to study microbial virulence and pathogenesis by such species as *Candida albicans, Cryptococcus neoformans* and *Enterococcus faecalis* (22), and has been used to study new therapies such as antimicrobial photodynamic inactivation (23, 24).

MECHANISMS OF PHOTOBIOMODULATION, MITOCHONDRIA AND PLASTIDS

It is widely believed that mitochondria act as the most important photoreceptor in mammalian cells (5). Pioneering studies by Karu and Pasarella (25, 26) established that cytochrome c oxidase (unit IV in the mitochondrial respiratory chain) absorbed red and near-infrared light, leading to increased electron transport, raised mitochondrial membrane potential and more oxygen consumption. Since mitochondria are critically important to PBM we will take a little time to discuss them in further detail. Lynn Margulis in 1971 proposed a theory that endosymbiosis played a major role in the evolution of plant and animal cells (27, 28). The idea was that mitochondria originated when a primitive eukaryotic cell engulfed an aerobic bacterial cell and the combination became stable (this was possibly a unique event), and in similar fashion, a primitive plant cell engulfed a photosynthetic blue-green algal cell that became a stable chloroplast. This theory was originally controversial, but began to be accepted when Schwartz and Dayhoff (29) compared protein and nucleic acid sequence data and produced evolutionary trees. The electron transport chains in bacteria are located in the plasma membrane, and are more complicated than those found in mammalian mitochondria. For instance, *Paracoccus denitrificans* is a soil bacterium classified as a Gram-negative facultative anaerobe. When this bacterium grows aerobically, its electron transport chain possesses four complexes that correspond to those in the mitochondrial chain. However, when this bacterium grows anaerobically with nitrate as its electron acceptor, the chain is structured quite differently. Electron transport chains in other bacteria vary in their cytochromes and can be branched. Electrons often enter at several points and leave via different terminal oxidases (30). Figure 1 shows similarities between electron transport chains in animal and plant mitochondria and in bacteria.

In plants, there exist several different organelles including mitochondria, and a range of different plastids. The most common plastid is the chloroplast containing chlorophyll for photosynthesis, but other plastids called chromoplasts, gerontoplasts, amyloplasts, proteinoplasts and elaioplasts also exist (31). All plastids, including chloroplasts, develop from undifferentiated proplastids and contain the same basic DNA. In addition, mature plastids are able to change from one type to another, as happens during the ripening of fruit, where chloroplasts (green chlorophyll) change into chromoplasts (red/yellow carotenes). Raghavendra and Padmasree discussed (32) how, although chloroplasts and mitochondria had been traditionally considered to be autonomous organelles, they might not be as

independent as they were once thought to be. The mutually beneficial interactions between mitochondria and chloroplasts, and also including the peroxisomes and the cytosol, appear to be in a delicate metabolic equilibrium. Mitochondria appear to be the key players because they function during both photorespiration and dark respiration.

The number of mitochondria inside different eukaryotic cells varies markedly from a single mitochondrion in some single-celled organisms (33), up to millions in a single oocyte of *Xenopus laevis* (34). Mitochondrial biogenesis is highly responsive to cellular demands for energy and also to environmental stimuli (35). The mechanistic target of rapamycin (mTOR) pathway regulates mitochondrial biogenesis to co-ordinate energy homeostasis with cell growth (36). PGC-1α (peroxisome proliferator activated receptor γ coactivator 1α) is a master transcriptional coactivator regulating oxidative metabolism in skeletal muscle (37, 38). PGC-1α interacts with Tfam (transcription factor A, mitochondrial) which is involved in the replication and transcription of mtDNA and the transcription of nuclear-encoded mitochondrial components.

The fission of mitochondria is correlated with mtDNA replication, and is controlled by Drp1 (a member of the dynamin family of large GTPases). Drp1 controls the final part of mitochondrial fission, pinching off the membrane stalk between two emerging daughter mitochondria. Several mitochondrial outer membrane proteins, including mitochondrial fission protein 1 (Fis1), mitochondrial fission factor (Mff) and mitochondrial dynamics of 51 kDa protein (MiD51), also known as mitochondrial elongation factor 1 (MEIF1), have been reported to promote mitochondrial fission by recruiting Drp1 (39, 40).

Mitochondrial fusion, on the other hand, is designed to avert and repair damage produced in mitochondria or mtDNA, by mixing the contents together so the undamaged structures predominate. The mitochondrial membrane fusion (MMF) family contains mitofusins, Mfn1 and Mfn2, GTPases localized to the outer membrane, and OPA1, a GTPase in the inner membrane that mediates inner-membrane fusion. Mutations in Mfn2 or OPA1 cause neurodegenerative disease.

There have been several recent reports that PBM has significant effects on mitochondrial dynamics including both fission and fusion (41–43).

PHOTOBIOMODULATION IN NON-MAMMALIAN HOSTS AND MODEL ORGANISMS

Table 2 presents a list of the non-mammalian organisms and model organisms that have been used in PBM studies. This is also graphically illustrated in Figure 2. The range of lifeforms that have been studied is impressive, but as yet, there are not many examples of each individual type of lifeform. Therefore, we have no idea as yet if the parameters described are the best for that particular indication or creature. The organisms range from the simplest prokaryotic unicellular bacteria (both Gram-positive and Gram-negative), to unicellular eukaryotic organisms such as fungi and paramecia. They go on to include, insects, aquatic organisms, worms and snails, amphibians and birds. Not only have laboratory studies used non-mammalian models to study scientific questions, but veterinarians have also employed

PBM in their clinical practice, in the case of aquatic species (44) and birds (45) (of course vets employ PBM on the vast majority of mammalian patients they encounter daily) (46).

Bacteria

Most investigations of red-to-NIR light irradiation of bacteria have been published in the Russian language. However, many of the findings have been summarized in English reviews authored by original investigators including many original papers (47, 48).

Tiina Karu was one of the first scientists to seriously study the phenomenon of PBM at a molecular and cellular level. There is a large body of work from Tiina Karu in Russia reporting on PBM effects on various microorganisms such as bacteria and fungi (49, 50). Karu group's investigations on bacterial photobiomodulation focused mostly on the *Escherichia coli WP2 trp*$^{-}$ strain. Most of the experiments were done with He-Ne laser (632.8 nm), but some experiments have also been reported with many other wavelengths in the 300–800 nm range, and also with 890, 950, 1066 and 1286 nm. The principal finding was that PBM causes a transient acceleration of cell division after moving the cells from the buffer in which they were irradiated to nutritive medium (latent period). The difference between irradiated and non-irradiated groups was notably diminished in cultures with only a short latent period.

In the *Anaerobacter polyendosporus*, PBM increased the number of germinated and outgrown endospores. A biphasic dose response was noted, the effect peaking at 300 J/m^2 (47).

It is noteworthy that the studies on the effects of PBM on *E. coli* were mostly limited to strains with impaired rates of growth (47, 51). Some experiments with wild type *E coli* failed to affect bacterial growth (52). Also, it has been reported that the PBM effects do not occur in the absence of oxygen. If anaerobically grown cells were exposed to oxygen for 24 hours, they again became sensitive to growth stimulation by PBM (53).

Some researchers have also used light in order to inhibit bacterial growth. For example, in one study, the effects of wavelengths of 660 nm, 830 nm and 904 nm were studied in *Staphylococcus aureus*, *E. coli*, and *Pseudomonas aeruginosa* and all of these wavelengths had inhibitory effects on bacterial growth (54). In another study, 810 nm light had an inhibitory effect on *P. aeruginosa* growth and a stimulatory effect on *E. coli* growth (55). In yet another series of experiments with *P. aeruginosa*, *E. coli*, and *S. aureus*, differing results were obtained with different wavelengths and doses (56).

In some experiments, red light has protected bacteria against ultraviolet radiation (57–59). Also, red light has been used for "mutagenic irradiation", meaning that it is possible to induce higher mutation rates in bacterial cultures by using the appropriate parameters of light irradiation (60, 61). This effect can be utilized in production of bacteria with specific metabolic traits. It is relatively easy to select the desired mutant form thousands of possible candidates.

Which mechanisms could explain the PBM effects in bacteria, especially *E. coli*? It has been shown that the PBM effects can be modified by quenchers and enhancers of singlet oxygen

($^1O_2$), and it was therefore suggested that PBM might generate singlet oxygen via excitation of cellular chromophores via a photodynamic effect (ie energy transfer between triplet states), and the damage caused by modest amounts of singlet oxygen could trigger the accelerated cellular metabolism of bacteria (53). Other groups had also suggested earlier that PBM using red-to-near-IR spectrum light happens in *E. coli* due to the absorption by the terminal respiratory chain oxidase complexes *cyt bd* and *cyt bo*, which have comparable functions to cytochrome c oxidase in eukaryotic cells (62). It has also been claimed that transient heating of microenvironment of these molecules might be involved (63). Most studies have been done with facultative anaerobes, but the Polo paper (53) also studied oxygen-free cell culture environment, suggesting that the PBM sensitivity began only after the bacteria had been for a few hours in oxygenated conditions "Moreover, cytochromes b and d are not expressed when *E. coli* is grown under anaerobic conditions, which would explain the lack of detectable photoresponse when the irradiations are carried out in the absence of oxygen, while the photostimulation reappears after the anaerobically grown cells are exposed to oxygen for a few hours, i.e. a time interval required for activating the normal oxygen-dependent metabolism." (53)

Fungi

The effects of PBM on fungi have not been much investigated. Some studies have been conducted in Troitsk, Russia by the Karu group. The original Russian language papers are difficult to access, but many experiments were described in two English review articles by the original researchers (47, 64). The majority of studies have been carried out on yeasts rather than on filamentous fungi presumably because of the relative ease of culture (54–56, 65, 66).

PBM of various strains showed a slight effect on the growth curves of yeast cultures. For example, irradiated *Endomyces magnusii* reached the stationary phase of growth 1.5 hours earlier than a non-irradiated culture (47). In *Torulopsis sphaerica*, the logarithmic phase of the growth occurred 1.5–1.8 times sooner than in the non-irradiated group.

The research was mainly undertaken with yeast strain *T. sphaerica* since it appeared to have higher sensitivity to PBM effects compared to other strains the group had been studying (*E. magnusii, Candida maltosa*, *S. cerevisiae* and *Saccharomycodes ludwigii*).

It was reported that He-Ne laser increased total cellular protein synthesis, and the biphasic dose response showed a peak at 460 J/m^2 (0.046 J/cm^2). The effects also included increased NADH dehydrogenase and cytochrome c oxidase activities and decreased activities of acid phosphatase and catalase. After these initial observations, ultrathin cellular sections of successive generations of *T. sphaerica* were prepared and examined using quantitative electron microscopy in order to analyze the structural changes in the yeast mitochondria. The findings showed that PBM with He-Ne laser was able to modify the ultrastructure so that giant mitochondrion could be observed in budding yeast cells after mitosis. PBM with the optimal dose increased mean mitochondrial profile area, while a higher dose led to an opposite effect, i.e. reduced mitochondrial area. PBM also increased the relative surface area of mitochondrial cristae by 25% according to the morphometric analysis. Moreover, PBM

increased the contact between mitochondria and the endoplasmic reticulum, which could reflect the increased mitochondrial capacity to uptake Ca^{2+}.

Some experiments with *Candida albicans* have also been published in other countries, and basically the results have been slight to moderate stimulation of growth (67, 68).

Paramecium

Paramecium sp are eukaryotic unicellular freshwater organisms with a characteristic slipper-like shape and are covered with beating cilia (69). Paramecia feed on microorganisms (bacteria, algae, and yeasts) and must therefore move around to force organisms, along with some water, through the oral groove into the mouth. The food then passes into the gullet. Moreover, paramecia can respond to stimuli by changing the coordination, direction and frequency of the ciliary beating, so they can "swim" away from danger.

Amaroli et al (70) used a flat-top hand-piece that could emit two different laser wavelengths, 808 nm and 980 nm (separately) at 0.1; 0.5; 1; and 1.5 W to generate 6.4; 32; 64; and 96 J/cm2 and examined swimming speed, intracellular calcium [Ca2+]i and nitric oxide (NO) immediately afterwards. Swimming speed was increased in a dose-dependent manner by both wavelengths with 96 J/cm^2 giving the best effect with 808 nm, and 32 J/cm^2 giving the best effect with 980 nm. After 64 J/cm^2 of 980 nm the Paramecia circled and showed avoidance behavior, while after 96 J/cm^2 was delivered the swimming abruptly stopped suggesting that the Paramecia were dead. NO was released by high doses of both wavelengths and [Ca2+]i levels were lowered by the two highest doses of 980 nm (64 and 96 J/cm2). In another study using this Paramecium model the same group has shown that 808 nm laser (1W; 64J/cm^2) increased cellular respiration in *P. primaurelia* inducing a 40% increase in oxygen consumption (71). A third study (72) showed the same parameters (808 nm, 1W, 64 J/cm^2) produced an increased cellular binary fission rate rhythm faster than the control cells. Yet a fourth study (73) used a different laser (1064 nm, 10 Hz, 100 msec pulse duration) delivering fluences from 30 – 90 J/cm^2. The best results were achieved with the lowest dose (30 J/cm^2 that increased ATP synthesis and fission rate rhythm. All higher doses induced adverse effects.

Plants

It is intrinsically difficult to study PBM in plants. This is because many wavelengths of visible light are absorbed by plants in order to carry out their normal physiological functions, the most important of which are photosynthesis and photomorphogenesis. Therefore, the default expectation is that plants will be stimulated by light. Pioneering studies in photosynthesis by William A Arnold (74) and KJ McCree (75) established the action spectrum for photosynthesis, which has a broad double peak in the blue (440 nm and a shoulder at 490 nm) and a double peak in the red (660 nm and 690 nm). The action spectrum drops off sharply above 700 nm (Figure 3).

Robert Emerson (Arnold's PhD supervisor) discovered the so-called "Emerson Enhancement Effect" that led to the realization that there were far-red absorbing chlorophyll derivatives (710–725 nm) that could mediate "uphill energy transfer" to photosystem II (PSII) and thereby enhance plant growth (76). Pettal et al reported the existence of even

longer far-red wavelength chromophores (up to 780 nm) that could in some circumstances stimulate PSII (77).

It should not be forgotten that plant cells contain two different light-responsive organelles i.e. chloroplasts and mitochondria at the same time. Chloroplasts and mitochondria were traditionally considered to be autonomous organelles but they are not as independent as they were once thought to be. Mitochondrial metabolic processes such as oxidative electron transport and phosphorylation, continue to be active in the light and are essential for sustaining photosynthetic carbon assimilation (78). The mutually beneficial interaction between mitochondria and chloroplasts is intriguing. The organelles within plant cells, including not only mitochondria and chloroplasts but also the peroxisomes and cytosol, appear to be in a delicate metabolic equilibrium. Disturbance of any of these compartments perturbs the metabolism of whole cell.

However, it is generally accepted that near-infrared light (> 800 nm) does not normally activate photosynthesis or indeed affect plant cells, possibly because plant biologists are largely unaware of the science of photobiomodulation. Johnson et al (79) addressed this issue by comparing oat seedlings grown either in the dark or under continuous illumination with NIR LEDs (880 nm or 935 nm). The power density was given in μmol/m^2/sec but it was approximately equal to 0.6 mW/cm^2. Illumination was constant from planting to harvest at 120 hours. They found the NIR illuminated seedlings had shorter mesocotyls (white part immediately after roots) and longer coleoptiles (part between mesocotyl and emerging leaf). Furthermore, the illuminated seedlings demonstrated shorter overall length but more vertical growth (gravitropism). The emerging leaves were longer. The authors attributed their observations to the presence of small amounts of red light impurities in their NIR LED emission spectra, presumably because they were unaware of PBM effects. They cautioned against the use of NIR imaging systems to monitor plant growth, because the imaging light could affect the growth.

Algae are metabolically similar to plants, but we have been unable to find any references to PBM in algae.

Some non-peer-reviewed comments on the website (“Hunker” https://www.hunker.com/) include statements such as “According to Texas A&M University, infrared light plays a part in the blooming of flowering plants. Plants grown indoors may grow well under fluorescent lights, but will not bloom until appropriate levels of infrared radiation have been introduced. This can be done using special horticultural lights, or simply by adding incandescent light bulbs” and “According to Planta, increased infrared waves can affect the speed at which plant stems grow. A short exposure to far infrared light increased the space between nodes when the exposure occurred at the end of an eight-hour light period.”

Insects

Bumblebees play an important role in pollination throughout the world, which is vital for ensuring that crops (and particularly fruit trees) continue to be productive. However the overuse of neonicotinoid insecticides has posed a threat to the worlds bee population (80). Neonicotinoids function as postsynaptic acetylcholine receptor agonists in insects, over

stimulating neurons and depolarizing the mitochondria (81). This neuronal damage to the insect brain results in immobility and subsequent death (82). Powner and coworkers in London reasoned (83) that using PBM to stimulate the bees' mitochondria might overcome the inhibition caused by neonicotinoids. The experiment comprised four groups with a 10-day treatment period and a 22 day recovery period: (a) controls, (b) neonicotinoid alone, (c) PBM alone, (d) PBM + neonicotinoid. Imidacloprid was administered in 50% sucrose solution, and 670 nm LED light was delivered for 15 min twice a day with a power density of 40mW/cm^2 from two light sources at either end of the 3L container. Imidacloprid caused the ATP levels to drop by 25%, but these were almost restored to normal by PBM. In the imidacloprid group 70 % of the bees had died by day 10, but in the PBM + imidacloprid group only 30% had died. All the imidacloprid group had died by day 20, but 30% of PBM + imidacloprid group were alive, followed by 40% of controls and 70% of PBM alone bees. Not only could PBM extend the lifespan of neonicotinoid treated bees but could also extend the lifespan of normal healthy bees.

Begum and colleagues studied whether PBM could extend the lifespan of aged *Drosophila* fruit flies. Seven-week old flies were either exposed to 670 nm LED light at 40 mW/cm^2 for 20 minutes once a day or else to normal room lighting (2 mW/cm^2). The survival of PBM flies was on average 10% greater than controls, and PBM treated flies contained 70% more ATP and 10% less complement component C3 (a marker of systemic inflammation) compared to controls. Moreover, PBM treated flies had increased mobility (climbing and distance traveled).

Aquatic organisms

Amaroli et al (84) studied the effects of PBM on the reproductive processes of sea urchins (*Paracentrotus lividus*). The justification for this study was the possible use of PBM in assisted reproduction and artificial insemination protocols in human fertility clinics. They irradiated *P. lividus* gametes, zygotes, embryos, and larvae and the fertilization rate and the early developmental stages were investigated. They used an 808 laser with a flat top hand-piece delivering a fluence of either 64 J/cm^2 at 1 W or 192 J/cm^2 at 3 W. The only difference was earlier development of fertilized eggs from irradiated sperm at the higher dose response (192 J/cm^2). They concluded that even higher fluences may be worth testing.

Milene Borges Campagnaro is a dentist who completed her MSc thesis in the Postgraduate Program in Dentistry at Catholic University of Rio Grande do Sul in Brazil in 2016 (85). The title was "*Embryotoxic and teratogenic effects of LLLT: a study in zebrafish*". She used two different lasers, NIR (830 nm, 90 mW, CW) and red (685 nm, 30 mW, CW) and looked at early stages (first five days) of development in the zebrafish embryos. Each laser was applied at a range of doses (0.5 J, 1 J, 2 J, 4 J, 8 J). The results showed that the PBM groups displayed a delay in egg hatching (eclosion), a higher mortality rate and some teratogenic changes were observed in the animals, as well as behavioral alterations, when compared to the control group. The heartbeat was higher in both IR and R groups, with the IR group showing the highest values. The cartilage and bone formation did not show any visual alterations. By and large this study must be considered negative, which might be due to the applied power being excessive.

Donald Stremme, a practicing veterinarian wrote a chapter entitled “ Laser Therapy for Aquatic Species” (44). Among the conditions that he discussed having treated were fish suffering from head and lateral line erosion (HLLE) disease and chronic ulcerative dermatopathy, long term lesions in a stingray, stomatitis in a reptile, spondylitis in various aquatic species, and improved bone healing in a dolphin with a fractured jaw.

Amphibians and Reptiles

Bibikova and Oron carried out a series of studies (86–89) on the use of PBM to regenerate a muscle injury in the European green toad (*Bufo viridis*). The motivations was that while PBM was known to help with muscle injury in small mammals which have a high rate of metabolism, would it also be active in amphibians which have a much lower metabolic rate. They first used a 6 mW HeNe laser to irradiate the cold-injured gastrocnemius muscle for 2.3 min every alternate day starting on the fourth day post-injury (88). At 9 days post-injury, mono-nucleated cells populated 70% of the injured area, but decreased more rapidly in the PBM-treated muscle than in the control. The fraction of the myotubes in the PBM muscles at 9 days was higher and at 30 days most of the injured zone was filled with mature muscle fibers. In the next study (88) they compared the HeNe laser with a pulsed Ga-As 904 nm laser (2.8 Hz, 200 nsec, average power 0.005 mW, peak power 9W). Both lasers stimulated muscle healing in the toads equally well and a single exposure on the 9th day was as good as five consecutive exposures every other day. In the 3rd paper (86) they looked at angiogenesis in toad muscles treated with the HeNe laser. The surface density of capillaries in the injured zone was 2.3-fold higher in the laser-irradiated muscles than in the control muscles at 9 days after injury, but the capillaries further increased in the controls between 9 and 14 days, while it declined in the PBM muscles. The surface-to-volume ratios indicated a straighter, rather than a convoluted appearance in the PBM muscles suggest a more rapid normalization. Finally in the 4th paper they looked at muscle regeneration following nerve damage (89). This time the muscles were surgically denervated rather than cold-injured. HeNe laser was delivered (6.0 mW, 31.2 J/cm^2) at 7 days post-denervation, and allowed better muscle regeneration with PBM.

Comelekoglu et al reported an electrophysiological study using frogs (*Rana cameroni*) (90). The skin was removed from the frogs and the sciatic nerve was exposed. Standardized needle electromyography and nerve conduction measurements were carried out using monopolar needle implanted into the sciatic notch. They used a Ga-As 904 nm laser (spot size: 0.28 cm2, pulse duration 220 nsec; peak power 27 W; fluence 0.0003–7.2 J). The pulse repetition rate was varied from: 1–1000 Hz to give average power values: 0.024–6 mW. The study failed to detect any significant effects of the laser on action potentials.

Cusack et al reported on a wound healing study in the green iguana (*Iguana iguana)* (91). Full thickness punch biopsy wounds were made, and wound healing was stimulated by 10 J/cm^2 660 nm laser better compared to the control treatment of silver sulfadiazine.

Worms and snails

Wu and Persinger (92) asked whether PBM could influence the regeneration of amputated planarian worms. The fact that planarian worms can regenerate their whole body if they are

cut in half has led them to be widely studies as models of stem cell mediated tissue repair (93). In fact, a planarian can regenerate its entire body from a piece that is less than 1% of the original body weight (94). Wu et al exposed whole and amputated worms to either ambient laboratory lighting, darkness, white (indium–gallium/yttrium–aluminum–garnet) LED, red (630 nm) LED, or near-infrared (880 nm) LED continuously illuminated for 3 days. LED irradiation used a power density of 1.2 mW/m^2 (0.12 $\mu W/cm^2$) for 24 h/day to deliver 0.01 J/cm^2 in total fluence each day. Compared to the other groups, amputated planarians exposed to NIR (880 nm) PBM displayed increased mobility by the 3rd day of exposure. Higher densities of stem cells were measured in these worms 84 h post injury.

Amaroli and coworkers studied the earthworm *Dendrobaena* veneta (95). While earthworms can still regenerate themselves after amputation (96), they are not as efficient at doing so as are planarian worms. These workers excised a piece from the caudal section of the worm and irradiated the "stump" with a CW 808-nm diode laser using a flat-top hand-piece at a power of 1 W to deliver a fluence of 64 $J/cm^{2.}$ After 24 h the worms were examined with histology and gross inspection. Immediately after PBM the worms showed a clear muscular contraction at the wound site and internalization of the alimentary tract only in the irradiated earthworms. At 24 h PBM treated worms had healed much better than controls, and there was a smaller coelomic plug in the irradiated samples due to muscular contraction reducing the leakage of coelomic fluid and of coelomocytes from the wound. Immunofluorescence showed less acetylcholinesterase staining in PBM treated wounds indicating an anti-inflammatory effect. In a follow-up study (97) using this model they confirmed their earlier findings of improved wound healing by PBM due to "effects on muscular and blood vessel contraction, decrement of bacteria load, reduction of inflammatory processes and tissue degeneration".

Natalie Duggett carried out her PhD research at Durham University finishing in 2013 (98). The title of her thesis was "*Photobiomodulation in Animal Models of Ageing and Alzheimer's Disease".* Duggett used 1072 nm LEDs at 5 mW/cm^2 to irradiate *C. elegans* and found the lifespan was increased by 16% after a 12-minute exposure to PBM. Control experiments established that the increase in lifespan was due to direct effects of the NIR light on the worms, and not on the bacterial lawn upon which they fed. Biochemical studies established the importance of pathways involving heat shock factor 1 (HSF1) and heat shock protein 70, that can function to prevent cell stress and lessen cell death.

On the other hand, De Magalhaes Filho and colleagues explored the effects of visible light on *C. elegans* longevity (99). On the surface, it may appear that their results were is contradiction with those of Duggett as they found that light reduced the lifespan of *C. elegans*. However, De Magalhaes Filho found the biggest effects with blue light (470 nm), a moderate effect with green light (530 nm), and only small effects with yellow (600 nm) and red light (675 nm), and did not test NIR light. They showed that visible light created photo-oxidative stress along with a general unfolded-protein response that decreased the lifespan of the worms. Long-lived mutants were more resistant to light stress, as well as wild-type worms that had been given antioxidants.

Contzen Pereira published (100) an interesting study in terrestrial pulmonates, a broad group of creatures including land snails and slugs. These mollusks are characterized by possession of a vascularized lung instead of a mantle cavity with gills. Snails are known to possess extraocular photosensitive neurons on the dorsal surface of the subesophageal ganglia that are proposed to be internal photoreceptors based on their electrophysiological properties (101). Based on the slow response time, Gotow and Nishi proposed that these neurons could not be involved in vision or image-formation and that therefore "we suggest that simple photoreceptors operate in the general potentiation of synaptic transmission and subsequent motor output; i.e., they perform a new photosensory function" (102). Pereira aimed to study the effect of light on cognition and behavioral response in the land snail *Ariophanta laevipes* (100). Snails were separately exposed to a 5 mW 630 nm laser held 1 cm above the brain region of the snail for 1 min 40 sec twice a day at 12 hr intervals (9am and 9pm) for a period of 5 days. Cognitive abilities of the snails were improved as measured by the time taken to complete the T-maze pre-and post PBM for 5 days. Memory-formation and retention and learning ability was improved by PBM.

Workers in Russia had tested various laser wavelengths on single isolated photosensitive neurons taken from snails (103). The preparation of the neurons was described here (104). Balaban et al (105) used a 10 mW HeNe (632 nm) laser focused into a small spot. They used an intracellular glass microelectrode filled with 2 M KCl to record the membrane potential threshold potential, resting potential, and neuronal spiking activity. When the spontaneously active neurons generating spikes every 7–10 min were irradiated in between the spikes, the depolarization of membrane and generation of action potentials occurred as a function of light intensity. The probability of spike generation increased until the intensity reached 1 W/cm^2. The depolarization had a threshold at 0.1 W/cm^2, then increased with increasing the intensity, and reached a plateau at 0.7 W/cm^2 (depolarization rate 0.18 mV/s).

Birds

The effect of light is very important in the poultry rearing and in egg production (106). "Photostimulation" (provision of ambient white fluorescent or incandescent lighting) is generally considered to be the cue to initiate puberty in chickens, although the response to light is readily modified by feeding (107). At photostimulation, light energy passes through the skull, and reaches the hypothalamus. When the hypothalamus receives a photostimulatory signal (sufficient number of hours of light above a certain threshold of intensity), hormones are produced that affect ovarian function (108). One of the first responses in the ovaries of the bird after photostimulation, is that the very small ovarian follicles begin to increase in size.

It has also been reported that illumination during the egg incubation period produced broiler chickens with improved health, productivity, and behavior (109). Conversely, eggs incubated in the dark hatched into chickens with a greater level of composite physical asymmetry considered to be an indicator of developmental stress. Sindhuraker and Bradley (110, 111) found that 24-hour white light illumination of incubated eggs led to chickens that could walk 2 days earlier than eggs hatched in the dark.

Buzza et al (112) in Sao Paulo Brazil, decided to test whether red light delivered during the egg incubation period could affect the hatched chickens better than bright white light. They found that the best results were achieved with low power densities (0.014 mW/cm^2) delivered continuously 24 hours per day for 10 days (a dose of 1.2 J/cm2 per day). Newly hatched chickens treated with PBM had a 25% increase in average size and > 70% increase in average weight compared to the controls. Figure 4 shows an example comparing the hatched chicks (controls, PBM every 3 days, PBM every day). There was no difference between a 630 nm laser and a 635 nm LED.

As mentioned above veterinarians are more likely than human physicians to employ PBM for many of the conditions they encounter daily in their practice. The book chapter “Laser Therapy for Birds” (44) listed the following conditions : Deep wounds; Feather disorders; Lacerations; Petagium restriction; Pododermatitis; Self-mutilation; Surgical incisions; Thermal burns; Arthritis; Articular gout; Edema; Fractures; Neuropathic pain; Muscular sprains and strains; Splayed legs; Wing tip trauma; Ascites; Gastrointestinal disorders; Renal disease.

CONCLUSIONS AND FUTURE DIRECTIONS

It certainly seems to be the case that all life forms can respond to PBM type interventions in one way or another. The reason for this is probably that all life forms rely on cytochromes for one function or another and most of them contain a respiratory chain either in the mitochondria, or in the plasma membrane in the case of bacteria.

During the preparation of this review article several points occurred to us that are worth mentioning. The first point is that the almost complete lack of studies of PBM on plant cells, is truly remarkable. The obvious explanation is that everyone assumes that red light drives photosynthesis, and cannot therefore carry out PBM. However, even with NIR light (wavelengths above 800 nm) which cannot carry out photosynthesis, but can stimulate mitochondria (at least in mammalian cells) there are virtually no papers. The one paper we could find attributed their results to “a small impurity of red light in their NIR LEDs” (79). Another point concerns PBM stimulation of bacteria. Despite the number of papers published there has not been much investigation of the molecular mechanisms involved. Considering the vast array of mutants now available for many bacterial species, this seems to be a missed opportunity. Reversal of chronological aging has become something of a “hot topic” in recent years. Much research into age-reversal utilizes model organisms such as Drusophila (113), *C. elegans* (114) or honey bees (115). In a similar vein, the study of epigenetics has become popular (116, 117) and there are few studies looking at the effects of PBM on epigenetics.

Considering the dramatic effects of red light PBM on hatching of larger chickens from eggs (112), there would appear to be many opportunities for PBM in poultry rearing and egg production, especially that now this is often conducted indoors under artificial lighting. Although much of the livestock industry is concerned with mammalian species and this may be off-topic, it is worth pointing out that there would appear to be large numbers of unexplored opportunities for PBM in agricultural food production.

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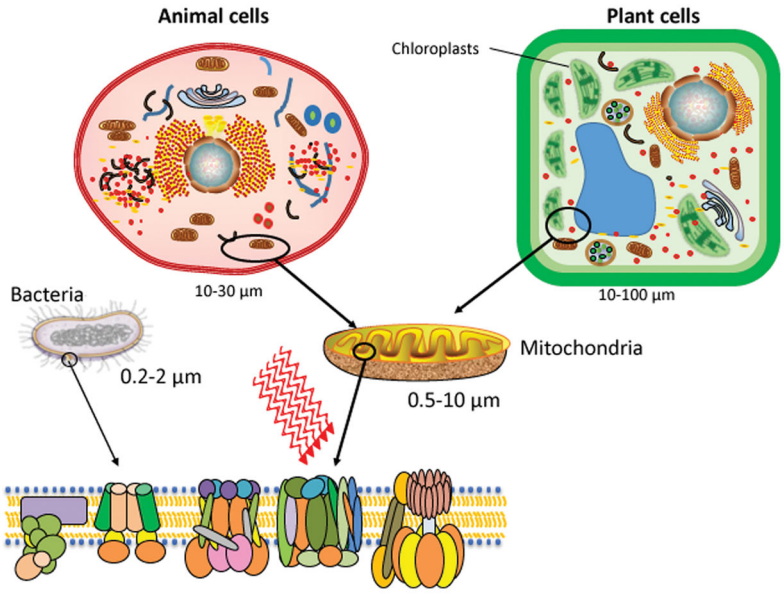

Figure 1.
Mechanisms of PBM in animal cells, plant cells and bacteria.

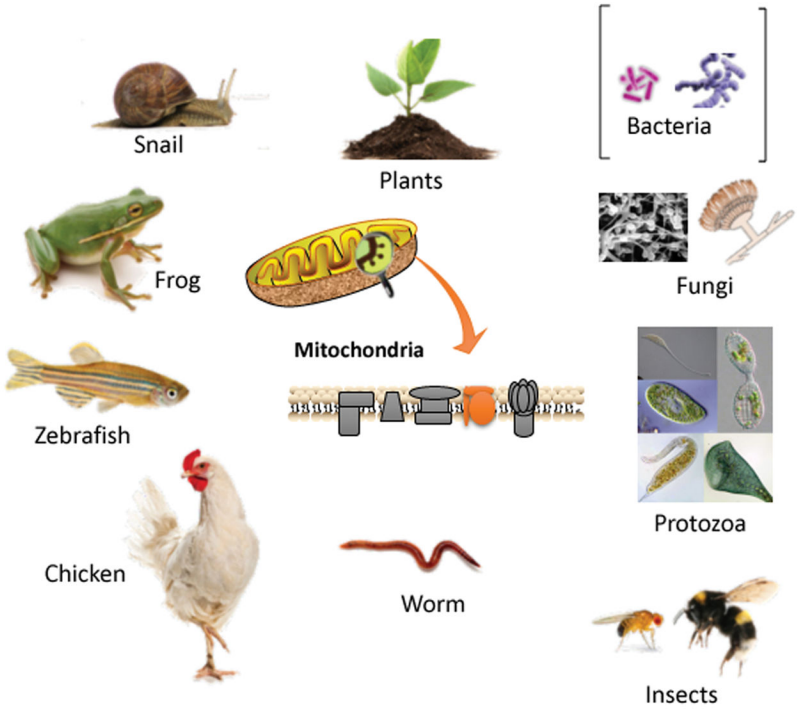

Figure 2.
The range of non-mammalian lifeforms which have been treated with PBM for various conditions.

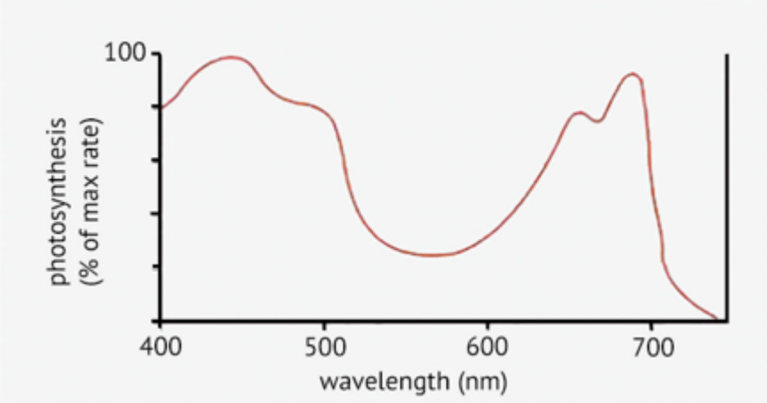

Figure 3.
Action spectrum for photosynthesis.

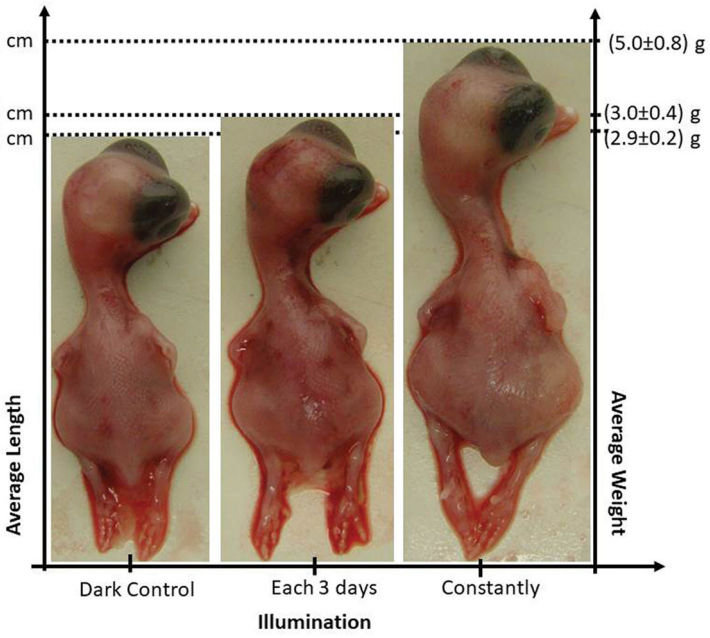

Figure 4.

PBM delivered continuously to eggs during the incubation period produces significantly larger chickens. Personal communication from Hilde Buzza, Sao Paolo, Brazil. Similar to a figure in (112).

Table 1

Advantages of model organisms compared to the mouse.

	Mouse	**Zebrafish**	***Drosophila***	***C. elegans***
Generation time	10 weeks	4–8 weeks	12 days	3 days
Numer of offspring	6–8 pups/2 months	200 eggs/week	120 eggs/day	140 eggs/day
Lifespan	2–3 years	2–3 years	40–50 days	14–21 days
Regulatory and ethics constraints	yes	no	no	no
Expense of housing	high	moderate	low	low
Number of neurons	> 70,000,000	~ 10,000,000	> 100,000	302
Fully annotated genome	yes	In progress/duplicate genes	yes	yes
Availability of mutants and transgenics	yes	growing	yes	yes
Homolgy with humans	> 90%	84%	77%	65%
Easy behavioral assays	no	yes	yes	yes
High throughput screening	no	yes	yes	yes

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Table 2

Non-mammalian animals and model organisms used for PBM.

Lifeform	Latin name	Function	Device/wavelength/dose	Author (year)	Reference
Bacteria	Various species *Escherichia coli*	Growth rate, mutagenesis	Laser, mainly 632.8 nm, 0.04–0.4 J/cm^2	Karu et al (1996) Nussbaum et al (2002)	(47) (55)
Fungi, yeasts	Various species *Saccharomyces, Candida*	Growth rate, metabolic activity	Laser, mainly 632.8 nm, 0.04–0.4 J/cm^2	Karu et al (1996)	(47)
Protozoa	*Paramecium primaurelia*	Increased swimming speed, ATP, fission rate, release of NO and stored calcium	Laser; 808 nm or 980 nm; 0.1; 0.5; 1; and 1.5 W; 6.4; 32; 64; and 96 J/cm^2	Amaroli et al (2015–2017)	(70–73, 118, 119)
Plants	*Avena sativa* cv Seger	Shorter mesocotyl; longer coleoptile; more vertical growth	LED/880 nm or 935 nm/0.6 mW/cm^2	Johnson et al (1996)	(79)
Bumblebee	*Bombus terrestris audax*	Reversed loss of ATP and mobility, caused by pesticide exposure	LED/670nm/12 hours daily for 10 days	Powner et al (2016)	(83)
Fruitfly	*Drosophila melanogaster*	Increased lifespan, ATP and mobility	LED/670nm/	Begum et al (2015)	(120)
Sea urchin larvae	*Paracentrotus lividus*	Earlier development of fertilized eggs from irradiated sperm (192 J/cm^2)	Laser/808 nm/3 W/64 or 192 J/cm^2	Amaroli et al (2017)	(84)
Zebrafish embryo	*Danio rerio*	Delayed hatching, increased mortality, teratogenicity, behavioral changes	Laser/685 nm, 30mW, 830 nm, 90mW/cm^2	Campagnaro (2016)	(85)
Aquatic species in veterinary practice	Fish, reptiles, dolphins	Ameliorations of skin lesions, stimulated wound healing, stomatitis, spondylitis	Various clinical laser systems	Stremme (2017)	(44)
Frog	*Rana cameroni*	Gastrocnemius muscle electrophysiology, sciatic nerve irradiated	Laser/904 nm, 220 nsec/up to 25.7 J/cm^2	Comelekoglu et al (2002)	(90)
Toad	*Bufo viridis*	Repair of muscle injury, enhancement of angiogenesis, and regeneration of nerves	Laser/632 nm/6 mW	Bibikova and Oron (1993–1995)	(86–89)
Green iguana	*Iguana iguana*	Full thickness wound healing was stimulated by 10 J/cm^2 better than control silver sulfadiazine	Laser/660 nm/5 or 10 J/cm^2	Cusack et al (2018)	(91)
Earth worm	*Dendrobaena veneta*	Improved healing and regeneration after amputation	Laser/808 nm/1 W/64 J/cm^2	Amaroli et al (2017, 2018)	(95, 97)
Round worm (nematode)	*Caenorhabditis elegans*	Increased lifespan mediated by HSF1 and HSP70 expression	LED/1072 nm/600 Hz/5 mW/cm^2	Duggett (2013)	(98)
Flat worm	*Dugesia tigrina*	Improved healing and regeneration after amputation. Increased stem cell proliferation	LED/880 nm/1 mW/m^2/0.01 J/cm^2	Wu et al (2011)	(92)
Snail	*Ariophanta laevipes*	Improved cognitive function and behavioral response as measured by T-maze	Laser/630 nm/< 5 mW	Pereira (2017)	(100)

Lifeform	Latin name	Function	Device/wavelength/dose	Author (year)	Reference
Snail	*Helix pomatia*	Single sub-esophageal neurons irradiated and action potential measured	Laser/632 nm/10 mW	Balaban et al (2017)	(105)
Chicken	*Gallus gallus domesticus*	Fertilized eggs hatched with bigger embryos	LED/635 nm/continuous	Buzza et al (2017)	(112)
Birds in veterinary practice	Various species of birds	Multiple diseases and conditions	Clinical therapeutic lasers	Ness and Meyer (2017)	(45)

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