Chapter

Firefly Translocation: A Case Study of Genetic and Behavioral Evaluation in Thailand

Anchana Thancharoen

Abstract

Conservation translocation is frequently used to conserve the threatened fauna by releasing individuals from the wild or captive populations into a particular area. This approach, however, is not successful in many cases because the translocated populations could not self-sustain in the new habitats. In this chapter, I reviewed the concept of translocation for conservation and the factors associated with the success rate. I used example problems from several cases involving different insect taxa. With its often high potential to mass rear in captivity, captive breeding can be a powerful tool by assuring large population size for insect translocation, which can result in a high success rate. However, genetic consequences from inbreeding and genetic adaptation to captivity can reduce the fitness of the captive population to establish successfully in the wild. Additionally, as the evidence in Japanese fireflies shows, the genetic differences between the translocated and local populations should be considered for a sustainable translocation program. A case study involved genetic and behavioral evaluation of *S. aquatilis* populations to assess the possibility of including the species for the firefly translocation program in Thailand. Although the results revealed no genetic variation among populations, examination of the variation in flash signals showed that the long-distance population had a longer courtship flash pulse than other populations in the Bangkok Metropolitan Region. With no geographical barrier, the light pollution and urbanization are probably important fragmented barriers causing adaptation of flash communication to increase the fitness. As a consequence, firefly translocation should consider flash variation between populations to prevent this potential pre-mating isolation mechanism from resulting in probable lower translocation success rates.

Keywords: Lampyridae, aquatic firefly, *Sclerotia aquatilis*, flashing behavior, population genetic, intraspecific variation, TiLIA software

1. Introduction

Fireflies have long been attracted the attention of people because of their fascinating flashing communication behavior [1]. In the past, firefly flashes on mangrove trees along the river were used as landmarks for boat navigation in the nighttime; while nowadays firefly habitats become "firefly tour sites" for nighttime activity and for supporting economic benefit to local communities [2]. Unfortunately, firefly populations decrease or disappear from many areas

worldwide due to habitat loss from growing of city developments, light pollution, water pollution and pesticide uses, which cause habitat destruction or fragmentation [3–7]. This same situation is faced by other insects [8]. In addition, firefly tourism without proper management could result in decreased firefly populations [2, 9, 10]. The problem has, thus, led to increased public awareness of firefly conservation.

Firefly conservation by reintroduced captive populations into the wild has received much attention. The successful captive breeding of some firefly species has intrigued numerous naturalists and conservationists including tourism stakeholders to plan to introduce captive breeding firefly populations into many areas to create firefly conservation sites, environmental learning centers and firefly tourism spots. The firefly mass rearing has been successful in some aquatic species, including *Aquatica leii* [11], *A. ficta* [12], *A. hydrophila* [13], *A. lateralis* [14], *S. aquatilis* [15, 16], and *S. substriata* [17]. A few of them have been used for conservation translocation. Many parks in Taipei, Taiwan were restored for suitable habitat and captive bred *A. ficta* fireflies were released [18–22]. In Korea, *L. lateralis* habitat (both running water and lentic water areas) was artificially created for releasing the mass reared populations of the species for ecotourism purposes [23]. As a symbol of nature in Japan, many firefly reintroduction and restoration projects of *L. cruciata* and *L. lateralis* have been done over the centuries, but not all of them have been successful [24]. Unfortunately, there are many cases showing strong ecological impact of introduced firefly populations on the native populations, which might eventually lead to the loss of the native populations in Japan [25]. This problem occurs where there is geographical isolation, based on examined differences of flash rate and genetic studies [26]. Therefore, the study of the impact of firefly translocation is essential prior to implementation of the program. Such impact studies have been lacking in Thai firefly translocation projects. Background information on genetic and behavioral variations among populations is necessary for development of a sustainable firefly reintroduction programs.

2. General aspects of translocations for conservation

Conservation translocation (population restoration) or called "ex situ conservation." Under the definition of the IUCN this is the intentional movement of released organisms from one to another site for conservation benefits [27]. That consists of two terms: (i) "reinforcement" which is augmenting a species where it already exists and (ii) "reintroduction" which is returning a species back to where it has disappeared [28]. With the increasing of habitat loss and fragmentation resulting in high species extinction rates and reduction of overall biodiversity, translocation of species may become an important management tool for recovery of the diminished or lost populations.

Many translocation programs have been carried out in many rare, threatened and keystone species to conserve species and genetic diversity. For example, European bison [29], Lake Sturgeon [30], Persian wild ass [31], green and golden bell frog [32], red wolves [33], and a few insects, (i.e., damselfly [34], field cricket [35] and fireflies [25]). Most of them have involved vertebrates, especially mammals and birds [36]. Consequently, translocation became an important conservation technique for birds in New Zealand [37]. However, as mentioned above, little work has been done in insect taxa.

The success of translocations was defined as resulting in self-sustaining populations in the release area. The success rate is affected by many factors. For example, species, habitat quality of the release areas, location of the release point, origin of

animals (captivity or wild), food habit (carnivore, herbivore and omnivore), clutch size, population density and competitors [36]. The research analyzed from translocation studies of 134 bird and 64 mammal projects concluded that the keys for high translocation success rate were releasing wild-caught animals, having herbivore food habits, releasing a large density, releasing in excellent quality habitats and releasing at the center of the area. In addition, the reproduction rate and generation length might affect the population sizes, chances of survival and genetic diversity of the target [38].

Many problems of population establishment from translocation were investigated. The small released populations might result in demographic and genetic consequences, for example, inbreeding depression [38]. Moreover, in the cases of releasing of a captive breeding population, the captive-born individuals provided from benign and stable breeding environments frequently have reduced fitness and high extinction rates after release into the wild. The physiological, behavioral and ecological problems from inbreeding depression, mutation accumulation, loss of genetic diversity and genetic adaptation to captivity were considered [39–43]. These could affect success of translocation programs through low adaptive potential to environmental changes [44]. Thus, many recommendations for dealing with the genetic issues are as follow: (i) minimizing numbers of generations in captivity, (ii) maintaining isolated captive populations with different genetic strains to reduce genetic load, (iii) allowing half-sib mating in captivity to reduce genetic adaptation to captivity and preserve genetic variation, (iv) minimizing kinship by equalizing family sizes and crossing, (v) observing the behaviors that might be lost in captivity, (vi) creating a rearing environment similar to the natural habitat to minimize the artificial selection, (vii) evaluating other risks (i.e., diseases), (viii) and collecting and analyzing long-term monitoring data routinely [39, 41–42, 45–47]. Although returning a lost species might not be same as the outcome of ecosystem restoration, the species perform ecosystem functions and generally relate to the other species. Polak and Saltz [48] suggested that the study on the effects of reintroductions on ecosystem functions should be integrated into the programs. Further, an overlooked issue of genetic impact is genetic contamination by maladaptive genotypes from reproductive crossing between genetically differentiated populations. That could push the recipient population toward extinction [49]. Therefore, the introgression with the population having local genetic makeup could result in a well-adapted population with similar morphological and ecological characters to local types.

3. Translocations in insects

The translocation of insects and other invertebrates has received considerably less attention than vertebrates; thus, not many examples of insects were translocated. However, ex situ conservation has become recognized as a more important technique for conservation for insects. With small body size, high reproductive rates, and short generation times, the insects have high potential to breed in mass captivity involving lower maintenance costs. Pearce-Kelly et al. suggested that the easy-breeding species with large captive populations have high potential for successful reintroduction programs [50]. The summary of 134 terrestrial insect translocations demonstrated that the proportion of success (52%) was higher than other animals while failed translocation programs were lower, 31% [51]. Thus, insects are the group most frequently considered in future translocations [52].

The objectives of insect translocation were classified into two groups, for conservation of the rare species and for socio-economic benefits of the flagship species.

Examples of the rare insect translocation are two vulnerable crickets, *Gryllus campestris* and *Decticus verrucivorus*, in England [53–54], the threatened tiger beetle *Cicindela dorsalis dorsalis* [55], a rare damselfly *Ischnura gemina* [56], Quino checkerspot butterfly *Euphydryas editha quino* [57] and the Genji firefly *Luciola cruciata* [58] (**Table 1**). With several iterations of releasing, the released insects could establish over a period of time and produced subsequent self-sustaining populations. The failure of translocation cases were caused by small released populations, disease infection, high dispersal stage used for releasing, low quality of habitat and weather conditions when releasing. The previous study [59] analyzed the documentations of 50 reintroduction activities of butterfly species and concluded that the successful projects had a higher number of attempts (per species) (11.1 ± 11.3 times for successful and 3.5 ± 3.2 times for unsuccessful programs). Successful programs introduced at least 292 individuals per reintroduction and continued for three years. Significantly, captive breeding was recommended for reintroduction programs for almost 50% of butterfly species.

As a dominant invertebrate flagship, the translocation of butterflies could be effectively used to build public awareness using live exhibits of butterfly farms. Many exotic butterflies were large-scale bred and imported across countries and regions for exhibition. If the butterflies come from similar environmental conditions and habitats, they might have high potential to establish in the new habitats. Consequently, the unintentional translocation might happen and cause ecological

Insects	**Threats**	**Sources of translocated population**	**Success?**	**Problems of the translocation**
Field cricket *G. campestris*	Rare and fragmented habitats	Captivity	Success (5 years)	- disease infection - cannibalism
Wart-biter bush cricket *D. verrucivorus*	Rare and fragmented habitats	Captivity	Failure	- high mortality rate in captivity result in small translocated population - high rearing cost
Tiger beetle *C. dorsalis dorsalis*	Sandy beach habitats of larvae were destructed from increasing of recreational activity.	Field collection (larvae)	Success (8 years)	- failure in adult translocation because of high dispersal behavior - larval predation by gulls
Damselfly *I. gemina*	Habitat structure changes and water area destruction from urbanization	Field collection (mating pairs)	Success (1 year in beginning phases)	- habitat changes from over vegetation in 2nd year. - unsuitable handling and marking techniques
Quino checkerspot butterfly *E. editha quino*	Habitat loss, fragmentation and extinction of native host plants	Captivity	Success	N/A
Genji firefly *L. cruciata*	Habitat loss, water pollution and tourism activities	Field collection and captivity	Success (70 years)	- harvested high amount of fireflies and released the non-native populations

Table 1.
Comparison of factors in some examples of rare insect translocation programs.

impact [60]. The opposite effect also may result, that captive bred populations lose the ability to live in natural habitats. After breeding in captivity for 100–150 generations, the large white butterfly have developed adaptive characters to captive conditions, i.e., heavier, higher ovary mass, higher numbers of laid eggs, and smaller wings that could decrease the butterflies' ability to re-establish in the wild [61].

The firefly is also a potential flagship to stimulate conservation awareness and action to support habitats for fireflies and other sympatric invertebrates. Apparently, firefly populations have declined or become extinct in many areas due to the impact of anthropogenic activities (i.e., habitat destruction, fragmentation, pollution and urbanization). Fireflies can be used to help promote public awareness and concern for biological diversity conservation.

The history of firefly translocation probably began in Japan [58]. The famous case happened in Tatsuno, Nagano prefecture where several thousand of the non-native Genji fireflies from Shiga prefecture were released as a tourist attraction. Subsequently the variation in flashing behavior and population genetics were investigated. Although the population of Genji fireflies in Tatsuno could self-establish over 70 years in the translocated area and bring more than 100,000 tourists a year, the native populations might be destroyed or lose genetic diversity. That is the risk under environmental change in the upcoming global crisis. Later, the scientists raised awareness of the firefly conservation issue and recommended the approach of using habitat preservation instead of artificial habitat creation for tourism. The fireflies were commonly labeled as an indicator species for environmental conservation. The translocation of captive fireflies in recovering polluted environments received more attention and resulted in appearance of 540 firefly events throughout Japan.

4. Genetic variation among firefly populations: the difficulty in translocation

Genetic issues become more important in sustainable biodiversity conservation especially in animal translocation. Avoiding or reducing genetic problems is a key to reducing the risk of extinction. Thus, not only focusing on maximizing species survival from established population measures, but also focusing on the genetic diversity, genetic drift and genetic adaptation to captivity are necessary to evaluate viability of populations in the long term.

The evidences of genetic and behavioral variation among firefly populations in Japan were discussed above. Firefly translocation requires an appropriate evaluation prior to their introduction into the wild. Likewise, the long term post-monitoring of both genetic and phenotypic measures is needed to measure the success of translocation and to identify future threats.

Genetic differentiation of fireflies is caused by various factors, including limitation of dispersal activity, habitat specificity or mating systems. The species with limited dispersal species have a higher probability of reproductive isolation. As in the desert firefly *Microphotus octarthrus*, which have winged males and apterous larviform females, the discontinuous habitats results in genetic isolation [62]. Strong habitat specificity was apparently involved, and there are several other cases of genetic divergence of fireflies influenced by geographical isolation. The variation of genetic structure of *Pyrocoelia rufa* in Korea was examined among islands, western and earthen parts being separated by mountain barriers resulting in different habitat types [63]. Consistently, the variation of genetic and phenotypic patterns of several firefly species in Japan was geographically separated by the Itoigawa-Shizuoka tectonic line. *Hotaria parvula* with morphological variation of body size

are associated with genetic differentiation and are reproductively isolated [64]. Likewise, two population groups of *L. cruciata* in eastern and western areas of the tectonic line were also genetically different and displayed different flash communication patterns (slow-flash and fast-flash types) [65]. The variation in male flash patterns (based on inter-flash interval) was subsequently confirmed to have the potential to hinder in pre-mating between populations. The intermediate flash type fireflies that might be introgressive hybridization were found near the barrier area [66, 67]. Surprisingly, the "quick-flash type" was investigated in the Goto islands, the western tip of Kyushu but it was in the same haplotype as the fast flash fireflies inhabiting the mainland [68]. On the other hand, *A. lateralis* populations throughout the Korean Peninsula, northeast China, Sakhalin, and Japan were examined for genetic variation of two flash pattern types (which also have a difference in adult emergence season duration) but they could not be separated phylogenetically [69].

5. A case study of genetic and behavioral evaluation of Thai firefly species, *Sclerotia aquatilis*

5.1 Background

Sclerotia aquatilis (*L. aquatilis*) [70] is an aquatic firefly species. Individuals are commonly found in freshwater habitats throughout Thailand, i.e., ponds, ditches, wetlands inhabited by an abundance of aquatic snails and aquatic vegetation such as duck weed, water lettuce, water hyacinth, *Typha* spp., water lily, and Indian lotus. It is a multivotine species appearing all year round with the life cycle duration of 3–5 months [71], **Figure 1**. The larvae live in the water by respiring mainly through a pair of caudal spiracles to receive the air from water surface. They are frequently found back swimming at the surface of water.

The species has high potential for reintroduction programs because of the successful rearing technique developed [15, 16] and their several adaptive characteristics that support recovery of the new populations in old/new habitats. Since *S. aquatilis* occurs throughout Thailand, the reintroduction programs are probably

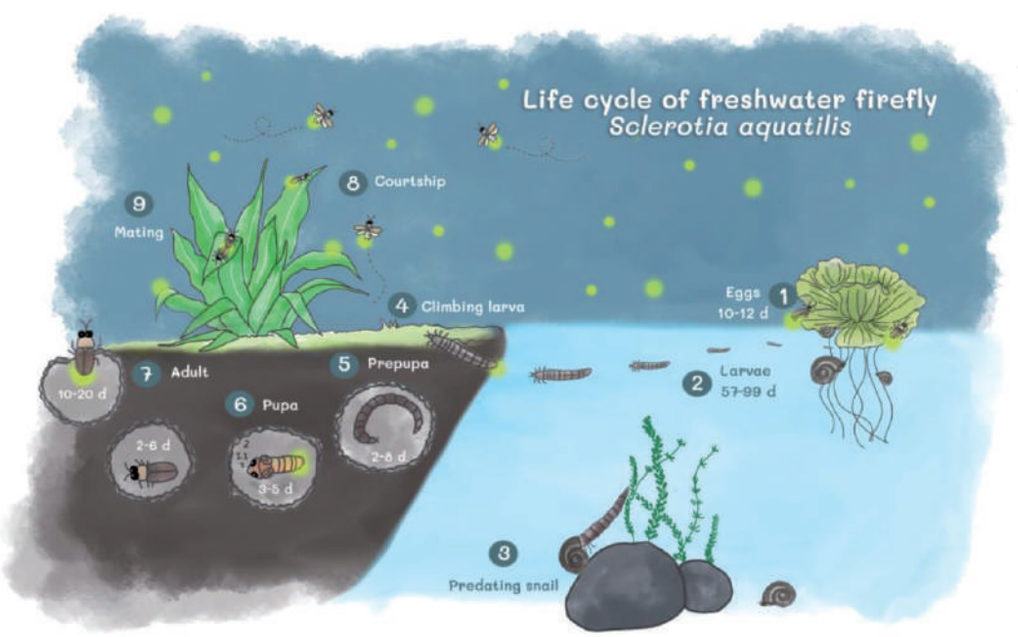

Figure 1.
Life cycle of S. aquatilis.

applied widely in the country. The firefly translocation has not previously been reported for this species.

There are many reasons suggesting genetic differentiation among *S. aquatilis* populations could lead to negative impact on translocation programs. Although geographic isolation frequently results in reproductive isolation by limiting gene flow between populations, it still remains unknown for firefly populations in Thailand. The expansion of cities and associated infrastructures not only destroy firefly habitats, but also creates habitat fragmentation. *S. aquatilis* populations are restricted to freshwater habitats, i.e., ponds, wetlands, and ditches. Adult female fireflies lack strong flight ability; therefore, habitat fragmentation seriously limits the range of their dispersal efforts, resulting in little immigration and even local extinctions. These limiting dispersal factors cause an increased the level of inbreeding and minimize interbreeding among spatially isolated populations. Thus, the probability of inbreeding and low genetic variability in nature is high in fragmented habitats. There is evidence of loss of genetic variation and the extinction of populations from habitat fragmentation in a butterfly metapopulation [72]. In addition, most *S. aquatilis* habitats overlap with human-used areas such as residential and agricultural areas, fireflies are subjected to many negative impacts from human urbanization, especially light pollution that can interfere with the sexual communication signals. Moreover, light pollution can be an effective dispersal barrier of fireflies. All these factors might result in both decreasing numbers and promoting inbreeding effects in populations.

5.2 Materials and methods

5.2.1 Study areas

During the process of urbanization, habitat loss and fragmentation have subsequently expanded particularly in Bangkok (BKK) area, where is the focus area for firefly reintroduction in this study. Historically, *S. aquatilis* inhabited in high abundance in the agricultural diches and ponds in the Chao Phraya delta area. However, the recent populations of the species have been decreased and become rare. The sources of translocated populations were from four nearby provinces, Samut Prakarn (SPK), Pathum Thani (PTE), Nakhon Pathom (NPT), and Suphan Buri (SPB) (**Figures 2** and **3**). Seven populations of fireflies from five locations were collected. One population from each province but two subpopulations from Pathum Thani (PTE2) and Nakhon Pathom (NPT2).

5.2.2 Firefly collection and maintenance

The collection of *S. aquatilis* specimens was conducted in all five locations during firefly season from August to November in 2012–2013, which was during the end of the raining season and the beginning of winter. The adult fireflies were collected at nighttime using a sweep net over freshwater areas. Adults were maintained in insect rearing cages supplied with a 10% honey solution on balls of moist cotton. In case of small populations, aquatic firefly larvae were also collected for molecular work. After observing the flashing behavior, the firefly specimens were placed in vials containing 100% ethanol, and stored in a − 80°C freezer prior the molecular study.

5.2.3 Genetic analysis

Genomic DNA from the hind legs of the adult specimens was extracted following the manufacturer's protocol using the DNeasy Blood & Tissue

Figure 2.
Map of Thailand the S. aquatilis study sites. The map illustration was modified from Vemaps.com.

Kit (Qiagen). A region encoding mitochondrial cytochrome c oxidase subunit II (COII) was amplified by the polymerase chain reaction (PCR) using the primers 5′-ATGGCAGATTAGTGCAATGG-3′ (TL2-J-3037) and 5′-GTTTAAGAGACCAGTACTTG-3′ (TK-N-3785) [69]. The PCR amplifications were performed as follows: an initial denaturing step at 94°C for 1 min, followed by 35 cycles beginning with a denaturation step at 94°C for 30 sec, an annealing step at 50°C for 30 sec, an extension step at 72°C for 1 minute, and a final step at 72°C for 10 min. The PCR product was verified by running through a 1% TAE agarose gel, stained with ethidium bromide and observed under UV light. The PCR product was treated with ExoSAP-IT PCR clean up reagent (Thermo Fisher Scientific, Massachusetts, USA) and sequenced by the 3130xl Genetic Analyzer (Thermo Fisher Scientific) with the BigDye Terminator v3.1 Cycle Sequencing kit (Thermo Fisher Scientific). The nucleotide sequences were assembled and edited individually using DNASIS Pro (Hitachi Software Engineering, Tokyo, Japan).

The numbers of base differences per site among sequences (p-distance) were calculated and constructed Unweighted Pair Group Method using arithmetic Average (UPGMA) tree using the p-distance by Molecular Evolutionary Genetics Analysis software (MEGA X) [73].

Median-joining networks among firefly haplotypes were constructed and post-processed under maximum parsimony in Network Version 4.6.1.1 (available at http://fluxus-engineering.com/sharenet.htm) to describe phylogeographic and genetic relationships between haplotypes.

5.2.4 Flashing behavior analysis

The live adult fireflies from each population were brought to the laboratory (26°C) for recording flash patterns within 1–2 days after collection to decrease the error from weakness and death. They were paired 1: 1 for mating in a mating arena that was prepared from a 7.1 × 11.0 × 6.5 cm of transparent plastic box with small moist cotton. They were allowed to have an adaptation period for 15–30 min before

Figure 3.
Habitat characteristics of the firefly collection sites, a) SPK, b) PTE, c) PTE2, d) NPT, e) NPT2 and f) SPB.

starting the experiment. The experiment was carried out under dark conditions (0 lux) for 30 min to 2 hr. after sunset.

The flashing communication was recorded using a Sony Handycam™ digital camera recorder (HDR-SR11E) at nightshot mode. All experimental mating boxes were separated from one another by placing black partitions between each arena to prevent flash interference from other mating pairs. Ten to 15 mating pairs from each population were randomly selected for video recording. Two flash types, courtship and warning flash types (**Figure 4**), which appeared at different periods of mating sequences, were recorded. The "courtship flashes" produced during courtship in responding to females, perhaps displayed during dorsal mounting. On the other hand, the brighter flashes displayed mostly during copulation called were defines as "warning flashes." At least 15 sec intervals or 30–50 flashes were recorded from each male. In case of small populations that had low numbers of females, the males were allowed to mate with virgin captive females to stimulate courtship behavior.

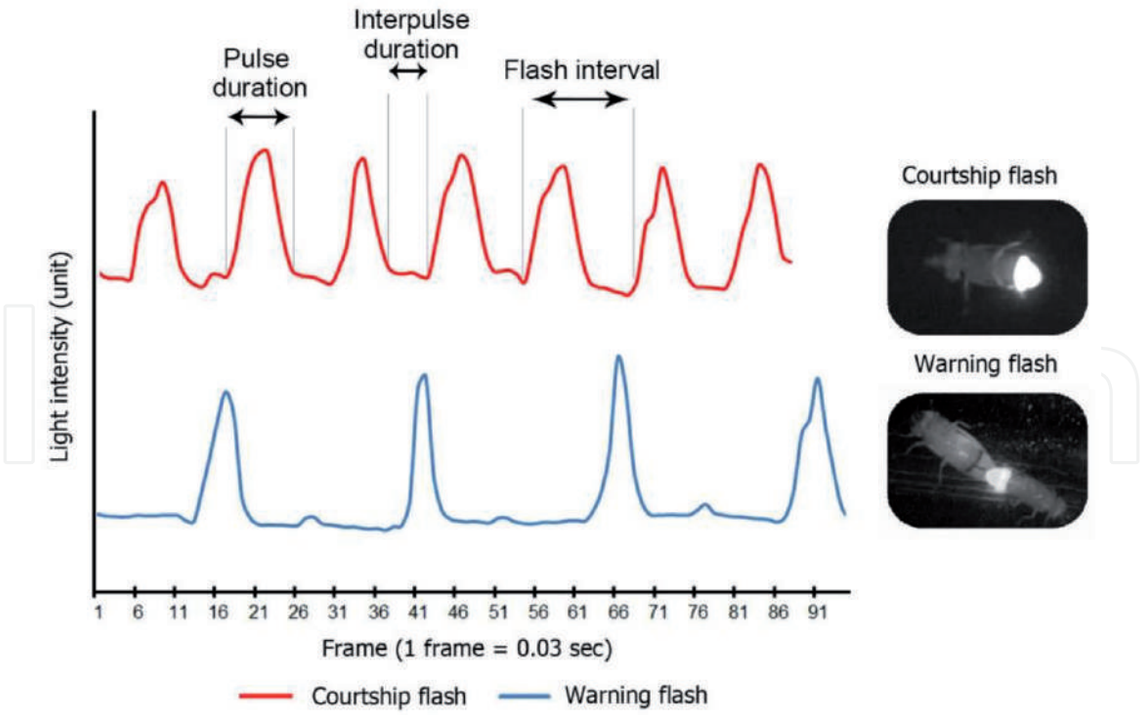

Figure 4.
Flashing behavior of male fireflies, courtship flash type (upper) and warning flash type (lower).

The video files were converted to audio video interleave or. AVI format files to analyze the flash parameters using time-lapse image analysis (TiLIA), a free software package for signal and flight pattern analyses of fireflies (available at Google Drive: https://drive.google.com/open?id=0B2o7FRVs2VohMmx2QzBVX3ZDeDA) [74] following the technique used by Thancharoen and Masoh [75]. The flash analysis was classified into three parameters, pulse duration, interpulse duration and flash interval, following previous study [76].

5.2.5 Statistical analysis

At least 30 flashes of courtship and warning flashes from each male were statistically analyzed. The pulse duration, interpulse duration, and flash interval among study sites were compared using One-way ANOVA and Tukey's multiple comparison tests. A value of $p < 0.05$ was considered statistically significant. The relationship between pulse and interpulse durations was tested using Pearson's correlation. All statistical analysis was performed using SPSS program version 24.

5.3 Results

5.3.1 Flashing behavior analysis

During mating behavior of *S. aquatilis*, the pulse durations of both courtship and warning flash types were quite similar, whereas the interpulse duration of warning flashes were twice longer than courtship flashes (**Table 2**). The correlation analysis of interpulse duration and pulse duration in each population showed that both flash parameters were negatively correlated (r in the range of −0.767 to −0.329, $P < 0.05$, n = 13). In case of short pulse duration, the interpulse duration was observed to be prolonged, stabilizing the flash interval.

The comparison of courtship flash parameters of all seven populations from five provinces showed that the fireflies from Suphan Buri province displayed different

Flash parameter	Duration in frame unit (mean ± SE)	
	Courtship flash (n = 60)	**Warning flash (n = 28)**
Pulse duration	5.54 ± 0.11	6.03 ± 0.17
Interpulse duration	6.78 ± 0.10	18.91 ± 0.34
Flash interval	12.32 ± 0.15	24.95 ± 0.38
Flash frequency	8.18 ± 0.09	4.03 ± 0.58

Table 2.
Flash parameters of courtship and warning flash types of S. aquatilis *(from overall populations).*

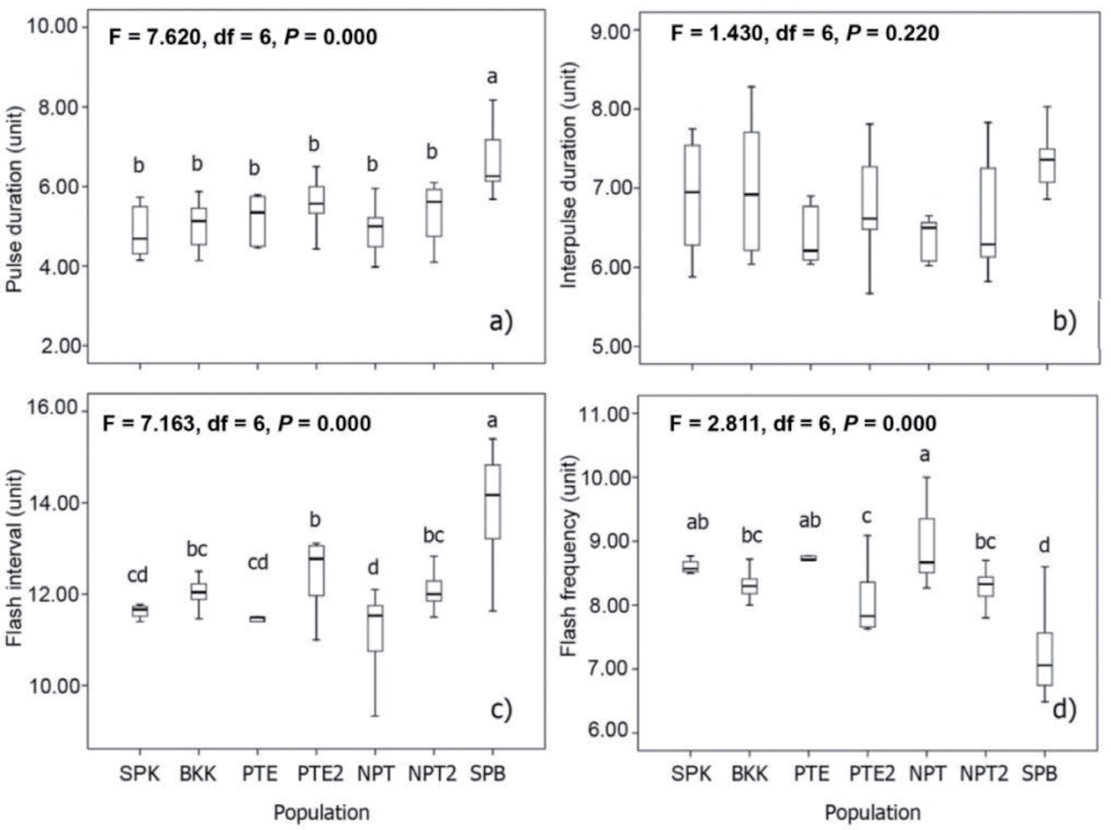

Figure 5.
The comparison of courtship flash parameters among seven populations of S. aquatilis; different letters indicate significant differences among different populations. Samut Prakarn (SPK), Bangkok (BKK), Pathum Thani (PTE), Nakhon Pathom (NPT), and Suphan Buri (SPB).

courtship flashes from the other sites located in the Bangkok Metropolitan Region (Samut Prakarn, Pathum Thani, Nakhon Pathom and Bangkok) (One-way ANOVA, $P < 0.05$; **Figure 5**). Results indicated that the Suphan Buri population had significantly longer pulse duration and flash interval resulting in slow flashing.

The flash parameters of the warning flash type could not be analyzed in all populations because not all experimental mating pairs displayed warning flashes. Therefore, only three populations from Pathum Thani, Nakhon Pathom and Suphan Buri province were analyzed. Perhaps because the mating happened under controlled environments without interference from mate competition and predation. Again, the Suphan Buri population flashed significantly differed when compared with other populations (**Figure 6**). It had a significantly long interpulse duration that resulted in having a long flash interval and a low flash frequency.

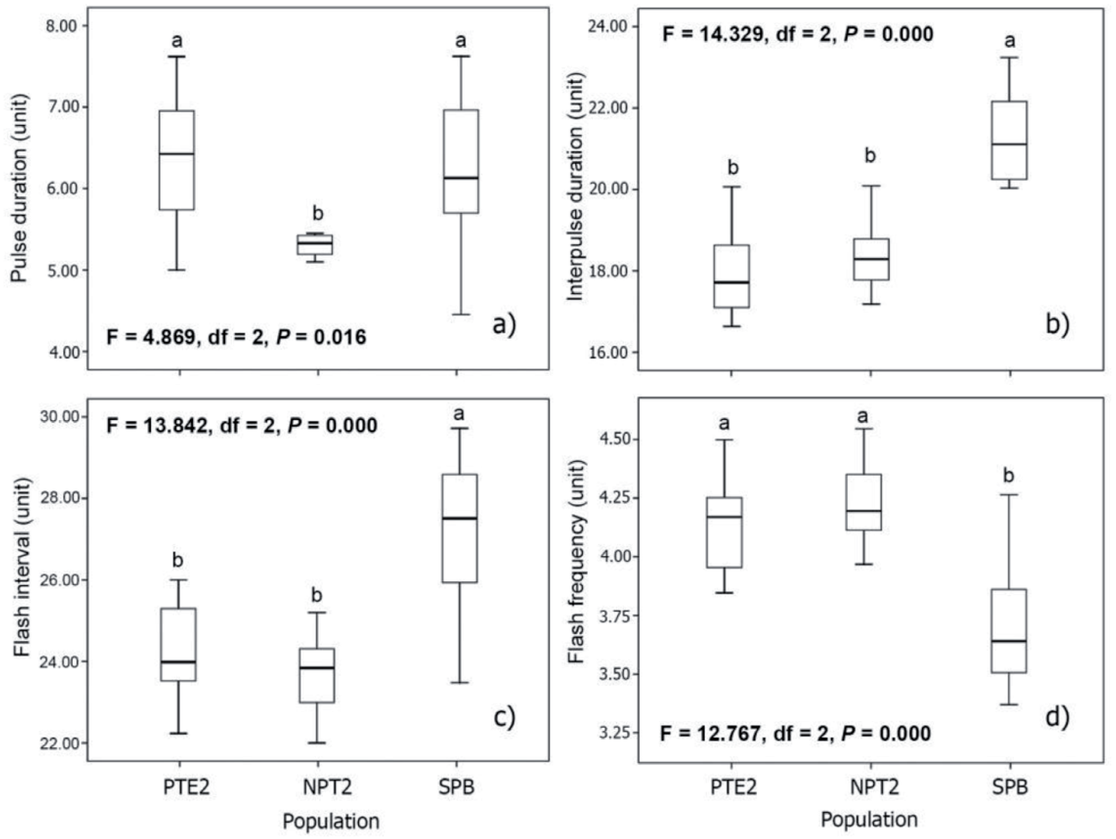

Figure 6.
The comparison of warning flash parameters among three populations of S. aquatilis; different letters indicate significant differences among different populations. Samut Prakarn (SPK), Bangkok (BKK), Pathum Thani (PTE), Nakhon Pathom (NPT), and Suphan Buri (SPB).

5.3.2 Genetic diversity of S. aquatilis

The genetic diversity of COII gene in *S. aquatilis* populations were examined from 132 individuals from seven locations in five provinces in the central part of Thailand. The sequences were registered in GenBank accession nos. MW800771 to MW800823 and MW814512 to MW814587. The p-distances among individuals ranged from 0 to 0.0122. The UPGMA tree revealed that regional cohesion of sequence types was not observed due to short p-distances (data not shown). The median-joining haplotype network was needed to confirm the low genetic diversity. The network revealed 37 haplotypes but not any phylogeographic sub-structuring of the firefly populations (**Figure 7**). Thus, no genetic differentiation was shown among the *S. aquatilis* populations examined.

5.4 Discussion

The study revealed flash signal variation among populations of *S. aquatilis* in the central part of Thailand. However, a distant population in Suphan Buri province apparently displayed longer pulse duration in the courtship flashes and longer interpulse in the warning flashes. As sexual communication, the pulse duration of the courtship signals is generally quite similar, preserving constant species-specific flash patterns. Most researchers studied "interflash interval" to define flash type from frequency, for instance, slow-flash, fast-flash, intermediate-flash and quick flash types [65–68]. However, the negative correlation between interpulse duration and pulse duration might help to balance the flash interval and flash frequency.

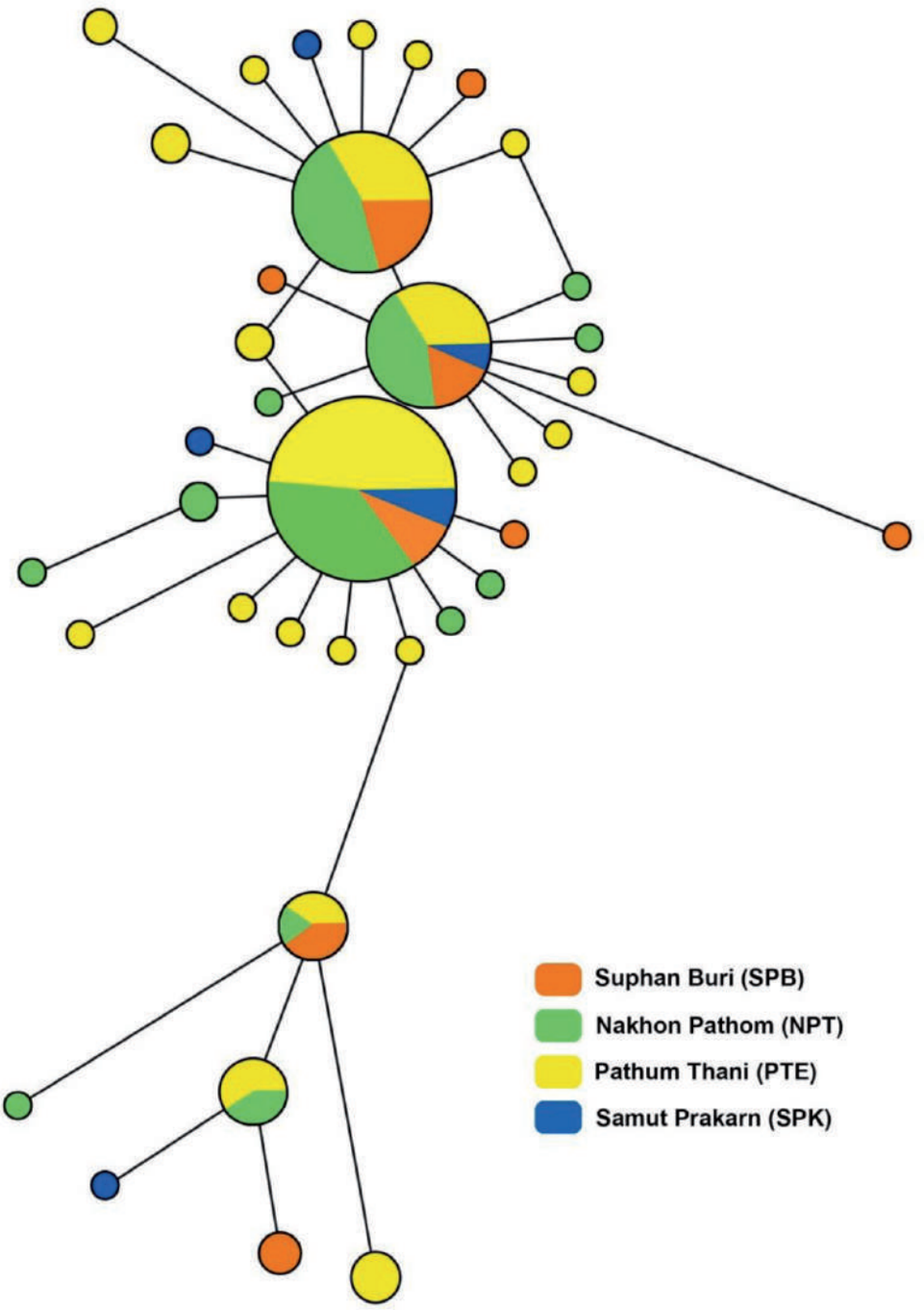

Figure 7.
Median-joining haplotype network generated from COII data from S. aquatilis collected from four locations in Central Thailand, different colors represent different collecting locations, sizes of nodes and pie segments are proportional to haplotype frequency, and length of branches is proportional to number of mutational changes between haplotypes.

Our finding was that there is intraspecific variation in flash communication of *S. aquatilis*. The fireflies in the Bangkok Metropolitan Region were fast-flash populations whereas the Suphan Buri population was slow-flashing although they did not show genetic differences among populations. This result is similar to the case of *L. lateralis* that *L. lateralis* populations distributed throughout the Korean Peninsula, Northeast China, Sakhalin, and Japan, the two flashing behavioral types could not be separated phylogenetically [69]. However, among populations with different flash types of *L. cruciata* in Japan, the genetic variation associated with flashing behavior was investigated [65, 67, 68]. The geographical differences caused by a great rupture zone of Japanese Islands might have had a strong

effect on this species. Similarly, as the most geographically distant location of our studied populations, the Suphan Buri population (109 kilometers from Bangkok), is probably isolated from the others. Although there are no geographical barriers influencing allopatric populations like in the Japanese case, habitat fragmentation including light pollution barriers probably significantly affect the firefly populations. *S. aquatilis* fireflies normally inhabit in or near freshwater areas, the active males can fly fast and travel a long distance, the inactive females remain near a water area. The reduced female mobility behavior might limit the dispersal ability of the species and result in population isolation. In addition, artificial night lighting could also interfere with flashes of *S. aquatilis* resulting in adaptive behavior to adjust their flashes.

The fireflies inhabiting the area of the Bangkok Metropolitan Region might face a habitat flooded with artificial light that causes reduced ability to communicate with their mates. Selection pressure favors adaptations of their flash pattern to minimize light competition or to increase the clarity of flash signals to improve their mating success. It might be possible that the environmental selection pressure happened in the fireflies. The plasticity of the flashing behavior depending on situation and environmental conditions were examined in many firefly species [75, 77, 78]. The fireflies in light polluted areas will modify their flash patterns to be faster to mitigate steady light from artificial night lighting. Similar adaptations occur in acoustically communicating animals, where ambient noise, especially anthropogenic low-frequency noise, affected acoustic communication in blackbirds [79], tree frogs [80], tree swallows [81], fish [82] and tree crickets [83]. The birds sing louder with higher frequencies to mitigate low frequency traffic noise, while the males of the tree crickets shortened their calls (echemes) and paused singing with a higher probability with increasing noise level without modification of song frequency or interecheme interval. Unfortunately, no work has been done on their genetic differences between the normal and noise polluted populations.

5.5 Recommendations

Generally, genetic differentiation among populations would happen in a heterogenous or mosaic environment by reduction of population size, genetic drift, gene flow and natural selection and accumulated by geographic isolation. Although there is no geographical isolation in the central region of Thailand, in case of *S. aquatilis*, gene flow is limited by the dispersal ability of adult females and aquatic larvae that are restricted to the aquatic ecosystems. In addition, the light pollution is likely an important barrier limiting the adult dispersal whereas habitat fragmentation reduces population sizes, reduces habitat size of firefly larvae and increases isolation of small subpopulations. The wild populations of the fireflies are at risk of extinction due to the effect of inbreeding depression.

The recommendation for *S. aquatilis* translocation is to consider: (i) no genetic differentiation between the local and the released populations, (ii) no divergence in flash signals to prevent pre-mating isolation between recipient and donor populations, (iii) the distance between populations might promote variation among populations; thus, closer populations are properly used for translocation, (iv) the sources of translocated populations come from a large population or several subpopulations to acquire proper numbers of source populations and decrease the effect of inbreeding depression. In addition, other factors, for example, habitat quality, source of translocated fireflies (from wild or captivity), released stage, frequency of releasing, released area and other environmental conditions during releasing, can relate to the success of program. This information is probably species specific; therefore, the biological and ecological characteristics of the focus species

are needed for translocation application. Significantly, the long-term monitoring of establish populations also is necessary.

In the case study, although the *S. aquatilis* populations in the central part of Thailand have no genetic divergence among populations, the variation of flash signals was found in a location of Suphan Buri province. The translocation of the species could happen if the donor and recipient populations come from Bangkok Metropolitan Region where the fireflies displayed similar flash signals and no genetic divergence among populations.

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Conflict of interest

The author declares no conflict of interest.

Author details

Anchana Thancharoen
Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand

Address all correspondence to: agrant@ku.ac.th

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Chapter

Semi-Intrinsic Luminescence in Marine Organisms

Jeremy Mirza and Yuichi Oba

Abstract

Light emission is widespread in the oceans, with over three quarters of all observed marine species exhibiting bioluminescence. Several organisms such as the copepod *Metridia pacifica* and the ostracod *Vargula hilgendorfii* have been proven to synthesise their luciferin and luciferase to facilitate light emission. However, many luminescent species lack the capability to do this and instead it is possible that they acquire some of the components for their luminescence through predation or filter feeding on organisms that produce luciferins or precursors to these molecules. This has resulted in many organisms using certain luciferins, such as coelenterazine, as their substrate without possessing a clear mechanism to synthesise these. This chapter will review several examples of these semi-intrinsic luminescent systems and how the substrates and enzymes can be obtained for these reactions. Moreover, it will look at why particular luciferins, such as coelenterazine, are more widespread and utilised in this manner compared to other substrates.

Keywords: Bioluminescence, Semi-Intrinsic, Luciferin, Coelenterazine, Imidazopyrazinone

1. Introduction

Bioluminescence is a chemical process numerous organisms utilise to produce light. This reaction has been studied in a wide range of taxa, in terms of its chemistry, evolutionary history and purpose in ecology [1]. This ability to emit light via a chemical reaction can be found in a diverse range of phyla, ranging from simple unicellular bacteria and protists to more complex organisms such as cephalopods and elasmobranchs [1]. Generally, this is a chemical reaction that involves the oxidation of a luciferin compound in the presence of a luciferase enzyme. This produces an unstable intermediate (usually a cyclic peroxide) that breaks down to produce a compound generically called oxyluciferin and gives off a large amount of energy as light [2, 3].

This phenomenon has evolved independently at least 94 and potentially over 100 times [4] across both marine and terrestrial genera, and around 80% of bioluminescent genera occur in the oceans [5, 6]. In marine ecosystems, it is estimated that up to 95% of organisms that dwell below 200 m depth are able to emit light [7–9]. Given the widespread utilisation of this phenomenon, there are a diverse array of luminescent systems that exist with several different substrates and a wide variety of associated enzymes.

Unlike the enzymatic component of the reaction where individual species are capable of expressing unique enzymes, luciferins are more conserved, and the same structures can be found across multiple distinct phyla. As of now at least 10 natural

luciferins have been identified in terms of their chemical structure [4, 10]. Of those, the four main marine groups of luciferins are bacterial luciferin, tetrapyrrole used by dinoflagellates and krill, cypridinid luciferin used by several species of fish and ostracods and coelenterazine which is used by luminescent organisms in at least 9 different phyla [11].

Despite being a critical component for light emission, many marine organisms do not produce their own luciferins, and obtain these small organic compounds from their diet by grazing or predating on other luminescent organisms [1]. These species exhibit semi-intrinsic luminescence, as they still express their own luciferase enzymes, however they can obtain the substrates and potentially precursors to luciferin needed for luminescence through their diets [12]. Some have even shown the capacity to obtain the enzymatic component of the luminescent reaction through their diet as well [13]. With regards to this phenomenon the most notable examples of semi-intrinsic luminescence involve coelenterazine and cypridinid luciferin [14].

This chapter will review the prevalence of known semi-intrinsic luminescent systems and how these organisms have attained light emission. Moreover, it will look at why these reactions and predator–prey relationships have evolved over time and discuss why certain substrates are more commonly observed in semi-intrinsic luminescence.

2. Sources of luminescence in semi-intrinsic systems

Identifying the presence of luminescence in an organism is well established and involves identifying the luciferin and luciferase involved in the reaction and separating them. The basic technique for luciferin and luciferase separation, developed by Dubois [15, 16] is termed "hot-cold extract". In this method, two water extracts of luminogenic tissue are prepared [3, 16]. The use of cold extract allows to preserve the activity of the enzyme (luciferase), while the heated fraction destroys the proteins and yields the luciferin, and when both extracts are mixed together an *in vitro* luminescence is produced [3, 16]. Each extract can be purified to allow for the identification of the amino acid sequence corresponding to the luciferase and the chemical structure of the luciferin [3, 17].

However, this in of itself does not establish how the luminescent organism obtained these components. A possible method to identify this is by constructing the transcriptome of an organism to prove the luciferase enzyme was expressed and not obtained through diet [3, 18]. However, this is a lot more difficult when it comes to identifying whether an organism can synthesise its own luciferin, as very few biosynthetic pathways have been established.

Despite this, it has been shown by controlling the diet of a number of higher taxa that their luminescence is dependent on the consumption of particular organisms [12, 19]. Subsequently, it has been possible to identify several organisms at lower trophic levels that can produce their own luciferin, including the ostracod *Vargula hilgendorfii* [20] and the copepod *Metridia pacifica* [14], both shown in **Figure 1**.

2.1 Cypridinid luciferin

Cypridinid luciferin was the first marine luminescent substrate to be identified in terms of its chemical structure. This compound was first isolated and crystallised by Shimomura and colleagues [21, 22], and the structure was determined by Kishi et al. [23], allowing for the detailed study of the biochemistry of this reaction [1]. The ostracod *V. hilgendorfii* was shown to secrete a luminescent mucus when disturbed, emitting a bright blue light at a peak wavelength of 453–455 nm [24]. The

Figure 1.
Photographs of luminescent organisms known to synthesise their luciferins. The ostracod Vargula hilgendorfii *(upper) synthesises cypridinid luciferin and the copepod* Metridia pacifica *(lower) synthesises coelenterazine. Photos taken by ken-ichi Onodera, and Yuichi Oba.*

luminescent cloud of mucus is emitted from specialised glands from two types of cell, one producing the luciferin and the other the luciferase [25].

Kato and colleagues [26, 27] showed that ostracod luciferin is synthesised from tryptophan, isoleucine, and arginine, via a currently unknown pathway. This was

observed by labelling the amino acid L-tryptophan with deuterium before feeding the ostracod *V. hilgendorfii* with this to confirm incorporation into the cypridinid luciferin [20]. *V. hilgendorfii* was shown to be the first example of a species that could use free amino acids to synthesise its imidazopyrazinone-type substrate, cypridinid luciferin. While this is used by several bioluminescent species, it makes up a small component of total systems in marine environments [20, 28].

2.2 Coelenterazine

The majority of luminescent organisms in marine environments with known or partially studied light emission systems utilise coelenterazine. Coelenterazine is an imidazopyrazinone compound (3,7-dihydroimidazopyrazin-3-one structure) that occurs exclusively in marine organisms in a wider range of phyla (at least nine) than any other luciferin [4]. These include radiolarians, ctenophores, cnidarians, molluscs, multiple arthropods, and some fish [29]. A large proportion of these organisms are assumed to have taken up this luciferin through their diet with only a few organisms shown to synthesise their own substrate [30–32]. The coelenterazine molecule was originally given its name due to the initial discovery of its presence in coelenterates, namely *A. victoria* and *Renilla reniformis* [33]. *A. victoria* is a hydrozoan jellyfish that emits a green light at 508 nm from a ring of photocytes on the peripheral regions of its umbrella [3]. Variants of this substrate exist in several species of squid either as a coelenterazine disulphate [34] or as dehydrocoelenterazine [35, 36].

Whilst coelenterazine has been found in a diverse array of phyla, a biosynthetic pathway and origin has not yet been determined for the majority of species, which are thought to obtain coelenterazine through their diet [12]. Coelenterazine has been shown to be synthesised in the deep-sea copepod, *Metridia pacifica,* via a similar mechanism to that observed for cypridinid luciferin in *Vargula hilgendorfii* wherein free amino acids are biosynthesised to form the coelenterazine luciferin [20, 26]. By labelling L-tyrosine and L-phenylalanine with deuterium it was proven that *M. pacifica* was able to incorporate these amino acids into its diet and that it was able to synthesise coelenterazine from two molecules of L-tyrosine and one molecule of L-phenylalanine [14]. Given that *M. pacifica* is at a lower trophic level it is likely to be predated upon by several higher taxa, many of which exhibit their own luminescent reactions [14, 37].

Recently it has been proposed that luminescent ctenophores are also able to produce their own luminescent components. The phylum Ctenophora or comb jellies are similar to the coelenterates in their morphology and apart from the family Pleurobrachiidae, all are presumed to be luminescent [38]. Ctenophores had previously been considered to be a source of coelenterazine synthesis in the oceans as there are reports of bioluminescence at early developmental stages [39]. When fed a coelenterazine-free non-luminescent diet, ctenophores were still shown to possess this substrate via mass spectrometry [40]. This recent study has implications that a number of other marine organisms, in addition to *M. pacifica* and Ctenophora, have the capacity to synthesise luciferin, which can provide a clear source of coelenterazine for a number of semi-intrinsic luminescent organisms.

3. Semi-intrinsic luminescent systems

3.1 Luminescence in fish

Most notable semi-intrinsic luminescence occurs in higher trophic levels such as among fishes. Several species have been shown to utilise the imidazopyrazinone type

substrates cypridinid luciferin and coelenterazine in luminescent reactions [1, 3, 41], though they are shown to express their own luciferase enzymes [6]. Often these have evolved to harbour luminescence in specialised regions of the body that allow for particular behaviours and functions for luminescence [1, 42].

3.1.1 Cypridinid luciferin in the midshipman fish

Several species of midshipman fish have been shown to utilise cypridinid luciferin as a substrate in their own luminescent reactions, despite showing no identifiable capability to synthesise their own luciferin [43]. A notable example of this has been observed consistently in the species *Porichthys notatus*, which can be found along the Pacific coast of the North American continent [44]. This species is characterised by an array of over 700 dermal photophores distributed along its head and body [45, 46]. Whilst light emission is restricted to specific organelle structures and can be stimulated mechanically, this is not sufficient to constitute a wholly intrinsic luminescent system. Moreover, non-luminescent individuals of the species have been identified when caught in the North Pacific off the coast of Oregon, where despite possessing the photophores in the same pattern, they did not exhibit luminescence [47]. This lack of luminescence was attributed to these animals not having a source of luciferin available from their diet at all of their life stages [48].

By adding small amounts of cypridinid luciferin to *P. notatus*, either by feeding them ostracods, or by intraperitoneal doses of as little as 6 μg of luciferin it was possible to induce luminescence [44]. This also was shown to be possible for completely non-luminescent individual midshipman fish and confirmed cross-reactivity of *P. notatus'* luciferase with cypridinid luciferin led to light emission [43]. It was identified that following consumption of ostracods, *P. notatus* is able to absorb the cypridinid luciferin through its gut. From here the substrate is believed to be able to bind non-specifically to erythrocytes in the blood plasma, possibly preventing autooxidation as it is transferred to the organelles of *P. notatus* where it can be oxidised in the presence of the luciferase enzyme to result in an emission of blue light [43, 49]. Light emission from the addition cypridinid luciferin to non-luminescent *P. notatus*, was indistinguishable from naturally luminescent Californian *P. notatus* [49].

The midshipman fish is a visually active nocturnal predator, that can utilise this acquired cypridinid luciferin to facilitate its hunting strategies. It has been speculated that the array of photophores on its body can mimic the light emission seen in euphausiid swarms, attracting unsuspecting prey [43, 50, 51]. This ability in combination with its highly evolved eyesight have allowed for it to be an effective nocturnal predator, feeding on both luminescent and non-luminescent organisms [52]. Cypridinid luciferin is not isolated to this species and has been found in several other luminescent coastal fishes including in the families, Pempheridae and Apogonidae [53]. Apogonids, or cardinalfishes are mostly reef dwelling with several species exhibiting visceral light organs that produce luminescence [54]. Similarly, Pempheridae commonly known as sweeper fishes, also have photophores along the length of their bodies and tend to be found in shallow marine and brackish waters [54]. It is likely that these species acquire their luminescence from ostracods, in a similar manner to the midshipman fish, though this is still to be confirmed experimentally.

3.1.2 Coelenterazine in Myctophid and Stomiid fishes

Cypridinid luciferin accounts for the luminescence observed in only a few species of bony fish as well as within ostracods, meaning it does not encompass a large amount of the total luminescence in marine environments. The most ubiquitous luciferin found in marine organisms is coelenterazine with species across multiple

phyla utilising this as their substrate for light emission [12, 40]. Among the fishes, numerous species of Myctophidae and Stomiiformes have been shown to utilise coelenterazine for bioluminescence, which is obtained through their diet, either by predating directly on coelenterazine producing copepods such as *Metridia pacifica*, or indirectly by predating on the consumers of these copepods [55, 56].

Myctophids, commonly known as lanternfish, are one of the most widespread and abundant families of mesopelagic fish in the oceans. They are distributed globally, with over 250 species identified across 33 genera and 2 subfamilies [56, 57]. Lanternfish are taxonomically distinguished by specific patterns of luminescent photophores that have allowed for a diverse array of strategies for both prey detection and predator avoidance [58, 59]. Generally, Lanternfish have two kinds of photophores, one along the body with the other proximal to their eyes (**Figure 2**). These two sets of photophores are able to illuminate independently from one another allowing for a variety of ecological functions. Photophores arranged on the ventral surface produce a constant dim blue luminescent glow and can allow for counter-illumination similar to other luminescent fishes, which would allow lanternfish to blend into the surrounding water column [56]. This would facilitate an ability to ambush prey as well as to hide from potential predators in the water column. These arrays of photophores form species specific patterns, which may allow for them to be used in intraspecific recognition [56, 60]. In addition to this array of photophores on the body, most lanternfish have one or more larger photophores on their head, usually positioned sub-orbitally or in the direct vicinity of their eyes [61]. Unlike the photophores on the ventral surface, these emit light in brief intermittent brilliant flashes. This is thought to allow either for predation by illuminating their prey, as well as being used to avoid predators by flashing and startling any larger organisms [56, 62]. Given that these suborbital photophores have sexual dimorphism, it is also possible that their main role is in communication within the species [56, 63].

Lanternfish feed predominantly on a variety of zooplankton including copepods such as *M. pacifica*, which would facilitate a source of coelenterazine luciferin for their luminescence, although it is difficult to assess this given the difficulties of maintaining deep sea fish such as myctophids in aquaria for sufficient amounts of time [55]. Lanternfishes are a major food source for a number of marine predators, including whales and dolphins. More importantly, they are also predated upon by squid and other larger lanternfishes, that also possess luminescence using coelenterazine or one of its derivatives [59]. Therefore, these potentially provide a key link in food webs by facilitating the transfer of coelenterazine from zooplankton to megafauna.

Stomiiform fishes include four families comprising of Gonostomatidae (bristlemouths), Phosichthyidae (lightfishes), Sternoptychidae (hatchetfishes), and the Stomiidae (dragonfishes) [64]. Among the dragonfishes, all species identified within this group have been shown to be bioluminescent, harbouring their light emission within specialised arrays of photophores. Apart from the Arctic Ocean, Stomiidae fishes are distributed globally, residing in the mesopelagic zone of the ocean between 200 and 1000 m depth, with some species recorded to a depth more than 4000 m [64, 65]. Luminescence may well be derived from the coelenterazine in their diets, with several species showing cross reactivity with coelenterazine in a similar way to some lanternfish [3]. However, it has been difficult to determine whether these animals are capable of synthesising their own luciferin, given that it is not yet possible to collect and maintain stomiid fishes in aquaria for any length of time. Dragonfishes are predators, utilising their bioluminescent emissions both as lures and as means to illuminate prey in order to facilitate prey capture [64]. Most feed on squid, shrimps and other fishes including lanternfishes, which may facilitate a source for coelenterazine in a number of these species [64].

Figure 2.
Photographs of Diaphus *sp. captured from a lateral (upper) and ventral view (middle). Displaying the photophores that produce a blue luminescent light (lower). Photographs taken by Yuichi Oba.*

Support for a dietary origin for luciferin in a number of stomiids is supported by their ability to uptake other small molecules to utilise in light emission. An example of this is shown in several species of loose-jaw dragonfish (*Malacosteus* spp.), that have a rare ability to emit longer wavelengths of luminescence that is red in colour, as opposed to blue light which is more ubiquitous in the oceans [1]. *Malacosteus* can also detect red wavelengths of light using a distinct mechanism requiring derivatives of bacteriochlorophylls *c* and *d* that enhance its sensitivity to these longer wavelengths [66]. As vertebrates are unable to synthesise chlorophyll, *Malacosteus* could obtain this through a diet, predominantly of grazers such as copepods that will contain phytoplankton derived pigments in their guts [64]. This strongly supports the concept that other small organic compounds such as luciferins can be taken up by dragonfishes, as well as other Stomiiformes to utilise in their bioluminescent reactions.

3.2 Other Coelenterazine utilising systems

Semi-intrinsic luminescence is clearly present in several marine vertebrates that utilise either cypridinid luciferin or coelenterazine as their substrate. However, this alone does not account for the diverse array of marine phyla that use coelenterazine in their bioluminescent behaviours. Many organisms previously considered to synthesise coelenterazine have since been shown to obtain this through their diet, including in the cnidarians where this was first discovered.

3.2.1 Cnidaria (Coelenterates)

Bioluminescence within the phylum Cnidaria has been studied more than in any other marine invertebrate. Most notably the hydromedusa *A. victoria* which emit light via the enzymatic oxidation of coelenterazine in the presence of calcium [12]. Unlike most coelenterazine utilising organisms that emit blue light, in *A. victoria*, light emission is green due to a green fluorescent protein. This emits green light via resonance energy transfer from the aequorin photoprotein [67]. According to Shimomura [3], photoproteins can be distinguished from luciferases by two general means, not requiring molecular oxygen for light emission and being capable of emitting light proportional to the amount of protein present [68]. Isolated aequorin can appear to emit light only by adding Ca^{2+}, and once the reaction is complete the protein does not appear to immediately be available for further reactions [69].

By controlling the diet of *A. victoria* in the lab it was possible to show that they are dependent on a dietary supply for their luciferin. When provided with an external source of luciferin to uptake after this, *A. victoria* was able to regain its luminescence [12]. The diet of *A. victoria* will consist of a variety of zooplankton, including luminescent copepods such as *M. longa* as well as luminescent ctenophores, which could provide a dietary source for their luminescence. Several other notable examples of luminescent coelenterates are presumed to obtain coelenterazine from their diet including the sea pansy, *Renilla* sp. and the sea cactus *Cavernularia obesa* [70, 71]. These anthozoans are found predominantly in tropical waters and may be able to obtain coelenterazine by feeding on suspended detrital matter that may contain the substrate.

3.2.2 Crustacea

Among the crustacea there is proven case of a fully intrinsic luminescent system in the copepod *Metridia pacifica*, and a probable case in the decapod shrimp *Systellaspis debilis* which appears to have the ability to synthesise the molecule from free amino acids [72]. Zooplanktonic species such as these potentially provide a source for a lot of the coelenterazine utilised in semi-intrinsic luminescent systems found in many marine organisms. However not all crustacea are able to perform this, and some such as the lophogastrid shrimp, *Neognathophausia ingens*, have been shown to require coelenterazine from their diet [31, 73].

These shrimp use bioluminescence to evade predators as they emit a brilliant blue cloud of luminescence when agitated that acts as a smoke screen [74]. Given that deep water visual predators have highly sensitive eyes, the bioluminescent ink cloud will have a much greater effect in startling nearby predators than the ink clouds produced by most cephalopods [75]. It is possible that producing this amount of luminescent material has a high energetic so it may be easier from an evolutionary perspective to obtain this through their diet instead of via an internal biosynthetic pathway.

3.2.3 Radiolaria

An assumption may be that as the majority of coelenterazine in the ocean is produced and utilised by eukaryotes, that organisms such as protists would synthesise their own source of luciferin rather than obtain it through their diets. However even protozoa such as several radiolarian species are not only capable of bioluminescence but obtain coelenterazine through their diet [1]. For example, bioluminescence has been found in several species of *Thalassicolla* and *Sphaerozoum* [29]. As protists they may appear to be unable to possess semi-intrinsic luminescence, however

these species are heterotrophic, and capable of consuming and digesting larger prey including zooplanktonic copepods [76]. As to the function of luminescence in these organisms it remains poorly understood, although given their dietary acquisition of luciferin, light emission may assist in prey attraction and capture [1].

3.2.4 Chaetognatha

Other smaller marine organisms are able to acquire luminescence through predation, such as at least two species of chaetognaths. This phylum comprises of small, elongated worms that are between 2 and 120 mm in length [77]. Commonly known as "arrow worms" at least two species have been shown to be luminescent and can be found at depths greater than 700 m in marine systems ranging from tropical to polar regions [78]. Luminescence in all of these species is emitted as a blue cloud of light and may facilitate a role in stunning their prey to assist with their hunting strategies giving the lack of visible light that will attenuate down to these depths. Despite being from evolutionarily distinct lineages within the chaetognaths, luminescent species such as *Caecosagitta macrocephala* [79] and *Eukrohnia fowleri*, have a relatively uncommon trait among chaetognaths, in that they have an orange-pigmented gut lining [80]. Digestive systems in semi-transparent organisms that are orange in colour, have the capacity to mask any luminescence produced by ingested prey [78].

This provides strong evidence that some species will predate on luminescent organisms such as copepods in order to provide a dietary source of coelenterazine for their luminescent reaction as shown in a number of other marine organisms [12, 48]. Once absorbed, coelenterazine would be able to be passed through to their luminescent organs that harbour the light reaction, which tend to be found on the lateral and dorsal fins as well as along the sides of the body of these species [78].

3.2.5 Ophiuroidea

Most species that exhibit semi-intrinsic bioluminescence acquire their luciferin via predation, most notably on luminescent copepods or on their predators. However, it is also feasible that filter feeders will be able to acquire coelenterazine and other luciferins through their diet. One such example is seen in the ophiuroids or brittle stars where many species have been shown to emit light [81, 82]. One such example is the brittle star *A. filiformis*, whose bioluminescence has been studied from a biochemical perspective for the past decade. This species feeds on suspended organic matter by extending its arms into the water column [83, 84]. Each of its arms are covered with light-emitting cells called photocytes that have been shown to be dependent on coelenterazine as a source of luciferin [81, 84, 85]. Additionally, the enzyme involved in its luminescent reaction was shown to be homologous to *Renilla* luciferase, which is a coelenterate also thought to acquire its luciferin from its diet [81, 86].

A recent study monitored *A. filiformis* kept in an aquarium for several months whilst controlling its diet [82]. Over five months a depletion in *A. filiformis'* luminescence was observed when fed a coelenterazine-free diet, strongly suggesting it acquired components for luminescence through filter feeding [82]. This was validated as there was a quick recovery in its luminescent capabilities once the brittle star was fed coelenterazine supplemented food. This animal signifies that semi-intrinsic luminescent systems are not simply found among tertiary consumers. This also supports the notion that numerous other filter and detrital feeding organisms that exhibit luminescence, acquire their substrates via their diet.

3.2.6 Tunicata

While it has not fully been confirmed yet, it is possible a large number of other filter feeding marine organism can acquire luminescent components from their diet. Within the chordates the subphylum Tunicata, comprises of a number of species shown to produce luminescence, although compared with other luminescent organisms these remain poorly studied. Within the tunicates, luminescence is well represented among the appendicularians with several species being confirmed to produce luminescence. One such example within this group is the larvacean *O. dioica*, which is a free-swimming tunicate that dwells in the photic zone of the ocean [87]. The animal has transparent body and a tadpole-like appearance throughout its life cycle, ranging in size from 0.5 to 1 mm. Light emission occurs as blue flashes of light from its body that can be induced by mechanical stimulation [88]. This animal has also been reported to emit light in the presence of coelenterazine, so it is possible that these are able to acquire coelenterazine from exogenous sources [87]. Larvaceans like *O. dioica* can secrete their luminescence as a mucus that will capture and collect particulate organic matter whilst the animals are filter feeding [89]. These secretions form luminescent "houses" or clusters of organic matter which can harbour all of the components for the bioluminescent reaction. On mechanical stimulation, these "houses" emit blue light showing that the components luminescence are all present in a way such that coelenterazine does not undergo autooxidation. This display of luminescence supports coelenterazine being utilised by this and other filter feeders for semi-intrinsic luminescence as stable luciferins can potentially be found in particulate organic matter that these organisms can feed on [87, 88, 90].

Another example of luminescence in tunicates is found in pyrosomes which are pelagic tunicates known for their sustained bright blue luminescence as well as their capacity to form sporadic and yet massive blooms such as those observed in this region [91]. There is currently a lack of consensus on the origin of luminescence in this species. A recent study has shown that light emission occurs in the presence of coelenterazine for the species *Pyrosoma atlanticum* [92]. Moreover, using transcriptomic analysis, an enzymatic sequence was identified as being similar to the luciferase found in the Cnidarian *Renilla reniformis* that uses coelenterazine as its light emitter. Subsequent expression of this gene showed that light emission occurred in the presence of coelenterazine strongly supporting that this is the luciferase involved in pyrosome bioluminescence [92]. Coelenterates and some echinoderms have been shown to utilise luciferases with a similar structure to *Renilla*, and a number of these are thought to acquire coelenterazine through their diets. Therefore, it is entirely feasible that pyrosomes such as this species attain coelenterazine through filter feeding, which may also occur for various other luminescent tunicates. However, it should also be noted that recent studies have identified and characterised potentially luminescent bacterial symbionts within *P. atlanticum* [93] which supports several previous studies on this system. Determining how this organism obtains its luminescence will rely on further confirmation what the source of light emission is in this tunicate.

3.2.7 Mollusca

Like previously mentioned phyla, some luminescent molluscs are able to acquire coelenterazine through their diet. This includes the clam *Pholas dactylus*, as well as several species of squid that have been shown to possess coelenterazine in their livers [94]. However, these animals do not use coelenterazine directly as their source of luciferin for bioluminescence. Instead, they use modified forms of this substrate, for example the firefly squid utilises a disulphate form of coelenterazine

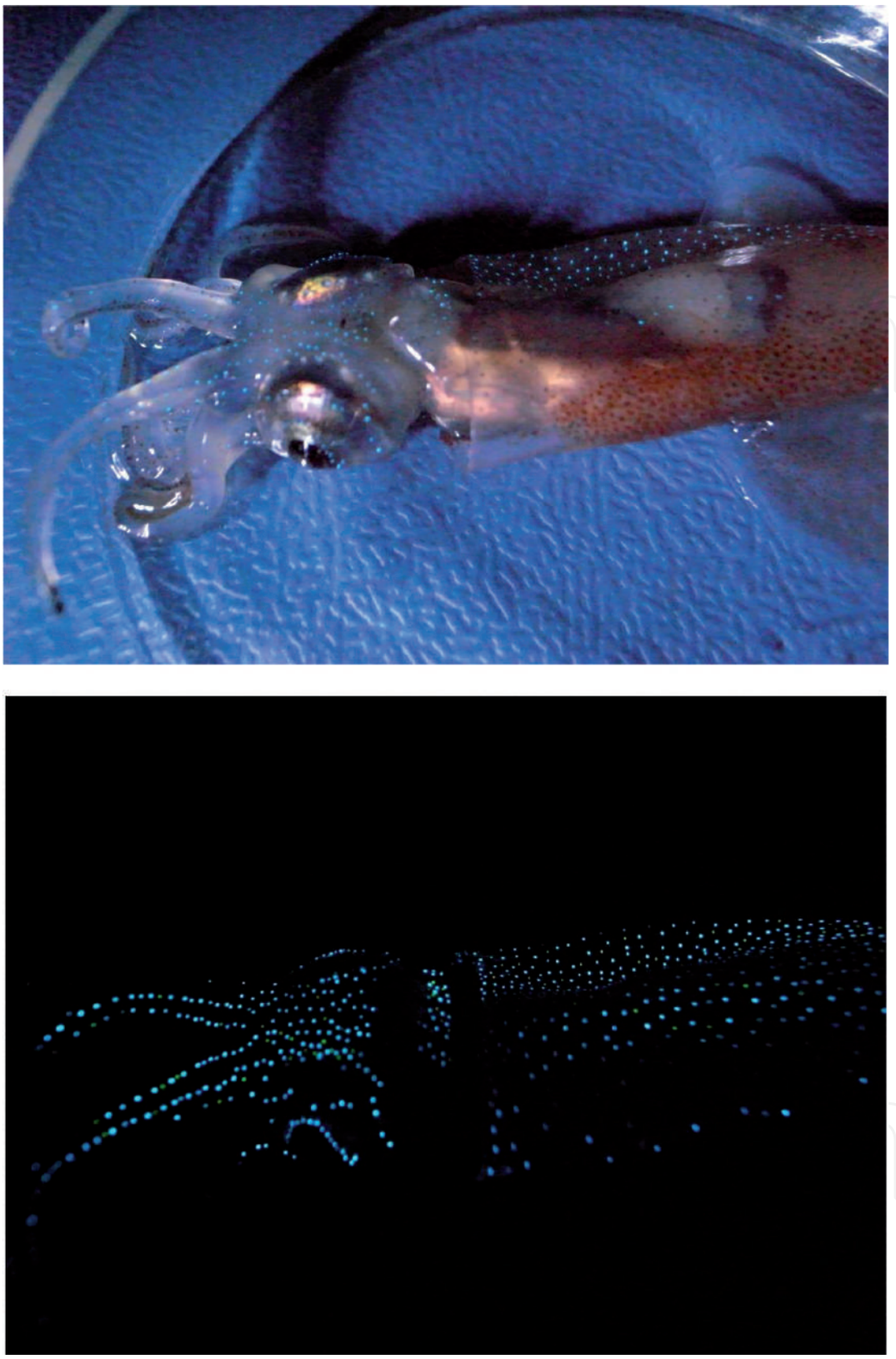

Figure 3.
Photograph of Watasenia scintillans *taken under natural light (upper) and in a dark room (lower) showing the luminescent photophores along its body. Photographs taken by Yuichi Oba.*

in its luminescent reaction [95]. These produce a dim continuous blue bioluminescence from ventral photophores, as well as a bright blue flash of luminescence (470 nm) from light organs on its arm tips after being mechanically stimulated [96]. The flashing ability may be used as a means of intra-specific communication and recognition although this has not yet been defined. The enzymatic oxidation of

coelenterazine disulphate [luciferin] in the presence of Mg^{2+} has led to emissions of blue light, however how or why obtained coelenterazine is modified remains undetermined [95, 97].

Another derivative found in several molluscs is dehydrocoelenterazine. This is an oxidised form of coelenterazine and was identified as the luciferin required in the luminescence of the clam *P. dactylus*, the purple back flying squid *Sthenoteuthis oualaniensis* and recently the Humboldt squid *Dosidicus gigas* [98]. In *D. gigas*, a blue bioluminescent light is emitted from an array of photophores on their body [39]. These structures are small, ovoid rice-like granules that are embedded in the muscle all over the squid on the mantle, fins, head, arms and tentacles [99]. It is entirely possible that this and other squids can obtain coelenterazine from lanternfishes which they are known to predate on. This coelenterazine may undergo an enzymatic oxidation to form dehydrocoelenterazine which is then utilised in its light emission (**Figure 3**).

3.3 Non imidazopyrazinone substrates

All examples of semi-intrinsic luminescence so far have involved either coelenterazine or cypridinid luciferin as the substrate. Dinoflagellate luciferin has also been shown be required by several heterotrophic organisms that appear to not be able to synthesise this luciferin. Dinoflagellates are unicellular organisms that account for the majority of bioluminescence observed in the surface ocean [100, 101]. The compounds involved with luminescence are regulated on a diurnal circadian rhythm, along with photosynthetic components. This means that dinoflagellates conduct primary production during the day and only produce bioluminescence at night, when this would be most effective. The structure of this luciferin was originally determined from *Pyrocystis lunula*. The compound is a linear tetrapyrrole which is very sensitive to non-enzymatic oxidation and is most likely to have derived from chlorophyll [102]. Within different species of dinoflagellates there is variation in the intensity and duration of light emission but in general light is emitted from organelles known as scintillons [101].

Dinoflagellate luciferin shows no similarities to other luciferins and is found in forms, one within dinoflagellates and another with two hydroxyl moieties in euphausiids (krill). This similarity suggests that there is some form of dietary link [102, 103]. Studies have shown luminescent euphausiids occurred in high densities which coincided with large populations of dinoflagellates during late spring [104]. Additionally, heterotrophic species of dinoflagellate, such as *Noctiluca scintillans* have been shown to feed on luminescent dinoflagellates such as *P. lunula*. When their diet was controlled in the lab to exclude luminescent dinoflagellates and all other phytoplankton, they were shown to lose their capacity to emit light [101]. Moreover, when fed other non-dinoflagellate phytoplankton, luminescence was maintained, suggesting that *N. scintillans* can synthesise the tetrapyrrole luciferin from chlorophyll [105]. These examples suggest other luciferins and their precursors may be taken up in the diets and utilised by consumers that already express the required luciferases for other non-imidazopyrazinone luciferins.

4. “Kleptoprotein” luminescence

A general consensus among semi-intrinsic luminescent systems is that the components of the light emission utilised by other organisms are the substrates rather than enzymes. As most of these animals acquire luminescence through their diets, any exogenous components would need to be able to withstand digestion and

potentially transport through the blood plasma to the luminogenic organs. Given this it seems unlikely that the enzymatic component of luminescence would be able to be obtained in this manner, as they would likely be denatured and completely broken-down during digestion [13].

However, a recent study on the *Parapriacanthus* fish, has shown that it is able to obtain both its luciferin and luciferase from its prey. Like midshipman fish, *Parapriacanthus ransonneti* predates on ostracods, which provide a source of cypridinid luciferin that is used in its light emission [13]. When *P. ransonneti* was fed on the ostracod *Cypridina noctiluca,* the luciferase identified from its light organs was identical to the luciferase of this species. When a different species of luminescent ostracod, *Vargula hilgendorfii* was identified in another individual fish, the identified luciferase was now the same as this ostracod, demonstrating the ability to specifically uptake luciferases from its diet to the fish's light organs [13]. Transcriptomic analysis of *P. ransonneti*, showed no transcripts corresponding to an ostracod-type luciferase, further highlighting that this was acquired via the diet (**Figure 4**).

This is the first reporting of this type of phenomenon in bioluminescence, and up until now it was assumed that any consumed luciferase enzyme would be broken down into amino acids or oligopeptides before being absorbed via the gut wall as nutrients [13]. However, the possibility of protein uptake without being fully broken down and retaining activity has been reported in several vertebrate immune systems. An example of this is seen in M cells within the mammalian intestinal epithelia as these have an important role in the immune system by transporting macromolecules and microbes into the cell via pinocytosis [106]. Similar examples of this have been observed in cyprinid fishes so it is feasible these or similar structures could facilitate the transfer of ostracod luciferase to the photophores of this animal [13].

This example of a "kleptoprotein" form of luminescence where both the substrate and the enzyme are provided through the diet, provides an additional novel category of luminescent reactions, as of yet not considered. Moreover, this highlights the possibility that other luminescent species may utilise this capability to obtain active exogenous luciferase from their gut. Potentially, this may include several species of fishes that predate on ostracods, whose light organs are often connected to their digestive tracts. This research may suggest that semi-intrinsic and "kleptoprotein" luminescent behaviours may be more widespread than previously considered, with proteins associated with other biological processes potentially being able to be attained via diet as opposed to gene expression.

Figure 4.
Ventral view of Parapriacanthus ransonneti *taken in a dark room to capture the light emission from these body regions. Photos by Okinawa Commemorative National Government Park (Ocean Expo Park), Okinawa Churaumi Aquarium.*

5. Why semi-intrinsic luminescence occurs?

Semi-intrinsic luminescence has been shown to exist in a number of organisms and is hypothesised to exist in several others. Cypridinid luciferin and dinoflagellate luciferin have been shown to be taken up by predators of ostracods and dinoflagellates respectively, notably several species of fishes, and euphausiid shrimp. However, the majority of semi-intrinsic luminescence, in addition to the majority of bioluminescence in the oceans involves using coelenterazine. Dietary uptake of coelenterazine has been shown in coelenterates, echinoderms, and decapod shrimp, while it is also strongly supported to be the source of luciferin in myctophid and stomiid fishes, chaetognaths, tunicates and several species of squid. Moreover, coelenterazine can be modified via oxidation or di-sulfonation, once it is taken up by species, allowing for a variety of different light reaction mechanisms to occur with this molecule. It is important to understand why some animals use semi-intrinsic luminescence, and the potential evolutionary origins of this, and how coelenterazine may spread across the food web and be the most common light emission system in the oceans. It is useful to consider whether this phenomenon along with "kleptoprotein" luminescence is a lot more widespread in other biological processes and systems.

There are two main groups of hypotheses on why bioluminescence evolved originally; one based around changes in the luciferin (substrate-centric hypothesis) [5, 107] and another that suggests changes occurred in what became the luciferase enzyme (enzyme-centric hypothesis) [108]. The first hypothesis suggests that the luciferin substrate evolved in order to protect organisms from reactive oxidative species (*e.g.*, hydrogen peroxide) in the water column [108]. Luminescent animal migrated to deeper water to evade visual predators and at these depths there was no longer significant oxidative stress. Therefore, the active selection pressure switched to the luminescent, communicative properties of luciferins, leading to more specific adaptations to predation, survival, and communication [1].

The alternate hypothesis focuses on the enzyme luciferase and that these molecules were originally less specific oxygenase enzymes [108]. The oxygenase enzymes mutated as a result of animals migrating to deeper waters to either evade visual predators, or to predate on organisms that have migrated to deeper water [5]. The mutation in oxygenase enzymes associated with display functions would result in external luminescence being exhibited [109]. These display pigments would previously have been associated with warning colourations or patterns to both recognise species and attract potential mates. There is evidence for enzyme-based hypotheses in terms of enhancement of visual signals [5]. However, there is no biochemical or genetic evidence that would support this hypothesis, and the mutation of the luciferase enzyme alone would not explain the convergent evolution of the bioluminescent reaction in multiple phyla [1, 5].

Whether one or a combination of both hypotheses are more viable for the origins of luminescence, both allow for the possible co-evolution of predators and prey that may utilise the same source of luminescence. Convergent evolution caused by environmental factors may have allowed for the presence of various enzymes that were compatible with the same substrate resulting in coelenterazine being utilised by both animals that can synthesise it as well as their predators. Moreover, given the energetic costs associated with synthesising luciferins, it may simply be more efficient for some of these organisms to acquire exogenous sources instead.

Semi-intrinsic luminescent organisms, particularly those that harbour coelenterazine, have shown the potential spread and dispersal across the food web for not just luciferins, but other molecules that may be involved in biological processes. A major source of coelenterazine is found in the copepod *M. pacifica* which is grazed

upon by a variety of organisms including coelenterates, lanternfishes, euphausiids, and radiolarians. Additionally, these animals, particularly lanternfishes are predated upon by tertiary consumers such as squid, stomiid fishes and luminescent sharks [110]. The consumption of copepods by zooplankton and higher taxa can lead to particulate organic matter or marine snow forming and descending to the depths of the ocean. These aggregates will contain detritus, plankton and larvacean houses, meaning that it is highly likely for free-available coelenterazine to be present. The coelenterazine within this particulate organic matter can then be taken up by filter feeders such as echinoderms and tunicates, allowing for them to utilise coelenterazine in their luminescent displays.

In a number of these organisms, luciferin has been identified in a sulfonated form. The most notable example of this is in the firefly squid, however sulfonated luciferins have been identified in *V. hilgendorfii* and *Renilla reniformis* [3]. This form is more stable than free forms of coelenterazine, and it is possible this is a stored form of luciferin that may prevent auto-oxidation that can occur. This more stable form may prevent breakdown and oxidation of the substrate when it is in the water column or during digestion. Potentially, a lot of these semi-intrinsic luminescent organisms will obtain their luciferins in this form, and then have the capability to de-sulfonate the luciferin to make it available for luminescence.

6. Conclusions

Luminescence has evolved and been prevalent in a wide variety of marine species being utilised for a number of predative, defensive and communicative functions. Some organisms have developed predator–prey relationships where the predator is able to acquire and utilise luciferin with its own luciferase to emit light. This chapter has reviewed many of the species that exhibit this type of behaviour and utilise semi-intrinsic luminescence, in addition to describing the sources of luciferin in these systems and how this molecule is able to be taken up by consumers. Although this has only been experimentally tested in a few species, it is highly likely that a number of other luminescent organisms utilise this, especially as it is a lot easier from an evolutionary perspective to obtain luciferins from the diet, compared with synthesising them from amino acids or other unknown biosynthetic pathways. This phenomenon raises the question of whether small molecules and enzymes involved in other biological processes are able to be taken up in this manner as well which could provide an evolutionary selection process that is an alternative to molecular evolution.

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Author details

Jeremy Mirza[1] and Yuichi Oba[2*]

1 University of Birmingham, United Kingdom

2 Chubu University, Kasugai, Japan

*Address all correspondence to: yoba@isc.chubu.ac.jp

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Chapter

The Ecology of Bioluminescence

Aditya Srivastava and Kalpna Katiyar

Abstract

Bioluminescence, or the ability to emit light biologically, has evolved multiple times across various taxa. As fascinating as the phenomenon is, various studies have been undertaken to harness this phenomenon for human use. However, the origins, distribution and ecology of bioluminescence still remain obscure. The capability to produce biological light is found in various species, ranging from tiny bacteria to huge fishes like lantern sharks. Many organisms that do not possess this ability partake in symbiotic relationships, resulting in a variety of anatomical and behavioral modifications. The ecological interactions resulting from bioluminescence are even more interesting and diverse, but many of them are still shrouded in mystery because of a lack of *in-situ* study. As agreed by many, bioluminescence conferred certain evolutionary advantages which still remain unclear. In spite of the lack of understanding, many spectacular ecological interactions like offence, defense, courtship or intra-specific synchrony have been observed, studied and documented, and their significance understood. As far as humans are concerned, efforts are being made to channel this capability to the best of our use, though some of these are still in their infancy. This chapter explores the origins, ecology and future prospects of bioluminescence in detail.

Keywords: Bioluminescence, Ecology, Bioluminescent organisms, Firefly, Deep-sea fauna, Fungi

1. Introduction

'Bioluminescence' refers to the phenomenon of chemically induced emission of light (or other electromagnetic radiations) by a living organism. It is a common occurrence frequently observed in various organisms, ranging from simple ones like bacteria to complex animals like deep-sea fish or fireflies, and even some fungi. The first accounts of bioluminescence are found in the works of Dioscorides and even Pliny the Elder, who believed that certain bioluminescent organisms had medicinal properties [1]. There are accounts of coal-miners using dried fish skins, and even bottled fireflies as safe light sources [2]. Charles Darwin also wrote about the glowing oceans in his travails. E. N. Harvey conducted extensive studies on this phenomenon, and wrote the first detailed account of all natural bioluminescent forms. In biochemical terms, the phenomenon of bioluminescence occurs due to an interaction of a substrate luciferin with an enzyme luciferase. Shimomura et al. were the first to obtain crystalline luciferin from the sea firefly *Vargula hilgendorfii* [3].

In this chapter, we explore the origins of bioluminescence in nature, its distribution, and the many ecological roles that it plays. Furthermore, the harnessing of this phenomenon for human use and the future prospects have also been discussed in brief.

2. The evolution of bioluminescence

Since bioluminescence has proven to be an energy-expensive process, the evolution of bioluminescence in nature must be of some ecological or biological significance, or must offer some evolutionary advantage to the organism. This is certainly true, because there are multiple incidences of the evolution of bioluminescence, all completely independent from each other, and showing a convergent evolution pattern [4, 5]. This trait is found in multiple species spanning different phyla. Some even show symbiotic association with microbes. All these species use this phenomenon for a diverse range of applications including evasion of predators, luring prey and even attracting mates [6–8].

Since bioluminescence is so widespread in nature, scientists have been speculating the cause of its origin and selection in the first place. The first speculation was made by E. N. Harvey himself, who believed that it had something to do with respiratory chain proteins, some of which may have had fluorescent groups or side chains [9]. Owing to the extensive research that he conducted, his theory gained some attention and credibility. It was, however, soon disproved. Some even state that bioluminescence may have merely evolved as a by-product of other metabolic functions, having no importance of its own. However, the repetitive and independent origins of bioluminescence in nature must mean that this trait does confer a significant evolutionary advantage to the species that exhibits it [10].

One theory, proposed by Seliger et al. in 1993, stated that luciferases were actually a group of mixed function oxygenases [11]. According to him, bioluminescence evolved primarily as a means of intra-specific or inter-specific interaction in the dark, deep sea biome.

Rees et al. conducted an independent study on coelentrazine, which is a marine luciferin [12]. They came to the conclusion that bioluminescence may have evolved as a biochemical pathway, mainly for the disposal of peroxide, superoxide, and other harmful oxygen species produced in the course of metabolism. This may have additionally been favored by the acute absence of illumination in the dark depths of the ocean. Bioluminescence may have undergone natural selection as these species may have progressed deeper in the dark depths of the ocean, where the selective pressure for anti-oxidant defense naturally subsided.

As is clear from the above discussion, there was a unanimous agreement among many that bioluminescence may have evolved in the deep sea ecosystem. Even today, the vast depth of the ocean abounds in various species that exhibit this trait. These may range from microbes like bacteria and dinoflagellates to complex organisms like crustaceans, molluscs, jellyfish, various bony fish, and even cartilaginous fish like sharks [10].

As of today, bioluminescence has many more purposes apart from free radical disposal, like camouflage, counter-illumination, warning colouration, predation or courtship, [10] which have been discussed in further subsections.

3. Distribution

As stated earlier, bioluminescence has emerged independently in nature on multiple occasions. Nearly 700 to 800 genera spanning 13 phyla, including both prokaryotic as well as eukaryotic species, have been reported to exhibit this trait [10, 13]. The evolutionary trends of bioluminescence show exemplary convergent evolution in many cases, because of the almost similar purposes this trait serves in various species, or because of the similarity in the biochemistry of the molecules involved.

Bioluminescent organisms are found in both terrestrial as well as aquatic habitats. However, the aquatic species are exclusively limited to marine ecosystems, and a freshwater bioluminescent system is yet to be reported [10].

For the sake of simplicity, the distribution of this trait has been discussed separately for bacteria, fungi and protists, and higher animals have been discussed separately.

3.1 Bacterial bioluminescence

It is a common belief that bacterial bioluminescent systems were among the first to originate in nature. Bioluminescent bacteria are present in both terrestrial as well as aquatic habitats, and can be found all over the world. In fact, these bacteria can easily be sourced from any tissue or detritus lying on beaches, or even from uncooked seafood [4]. The glowing oceans, which are a spectacular result of these microorganisms, have been described in detail in the travails of Darwin, and can be observed, or rather enjoyed at various locations all over the world.

Bioluminescent bacteria mainly belong to the class *Gammaproteobacteria*, and are confined to three genera, namely *Vibrio*, *Photobacterium* and *Xenorhabdus*. Out of these, *Vibrio* and *Photobacterium* are mostly found in marine ecosystems, whereas *Xenorhabdus* inhabits terrestrial habitats [14]. New strains of bioluminescent bacteria are still being discovered [15]. A remarkable fact about bacterial bioluminescence is that all bacterial bioluminescent systems are exactly alike in terms of biochemistry, i.e., they all rely on flavin mononucleotide (FMN), myristic aldehyde and NADH, and also oxygen [16].

Bioluminescent bacteria may exist as free-living, symbiotic or even pathogenic forms. However, a completely obligate bacterial symbiotic system is yet to be observed in nature [8]. For example, *Vibrio fischeri* has been known to colonize specialized "light organs" [17] in the fish *Monocentris japonicus* [18], and also exhibits mutualistic relationship with Hawaiian squid *Euprymnia scolopes* [10, 14], and various species from the genus *Photobacterium* have been known to exhibit symbiosis with various fish, molluscs, etc. [19] and even cause diseases in some others [8]. However, there has been no genetic alteration in the bacterial genome for the said symbiosis. Though the animals showing the said symbiosis have developed exclusive modifications like light organs, they do not show any endosymbiotic behavior. The development of the said specialized organs may even be influenced by the presence of the symbiotic bacterial population [4]. One hypothesis accounts for the emergence of bioluminescence in bacteria because it promotes such symbiotic behavior, conferring a survival advantage to the microbes [10]. The symbiotic behavior may further be promoted because of the fact that the luminescent machinery of the bacteria is instrumental in getting rid of the reactive oxygen species produced in the host tissue [20]. The symbiotic microbes are obtained externally, and the hosts show some degree of selectivity towards the symbiont [8]. It appears that the host organisms 'choose' the colonizing symbiont according to the availability as per the depth which they inhabit. Furthermore, the said hosts can even dump the symbiont cells in order to keep their population in check [20].

Terrestrial bioluminescent bacteria are rare, and are known to infect nematodes that parasitize glowworm larvae. Upon the death of the larva, predators and scavengers ingest the carcasses, hence dispersing the bacteria as well as the nematode. Other than that, bioluminescent bacteria have been observed to inhabit various depths of the ocean, and are found even in sediments, seawaters, saline lakes, etc.

3.2 Fungal bioluminescence

Of all the bioluminescent systems that have been studied, fungal bioluminescence remains by far the most poorly investigated of them all, even though fungi

are the only terrestrial eukaryotes that exhibit bioluminescence, besides animals [10]. This might be owing to the fact that most initial attempts at determining the enzymatic nature of fungal bioluminescence were failures, and have only recently been confirmed successfully [21]. The study of fungal bioluminescence has thus gained sudden prominence [22], and a genetically encodable bioluminescent system for eukaryotes has been developed [23]. Kaskova et al. conducted an extensive study of the fungal bioluminescence and colour modulation mechanisms [24].

Out of all the fungal species that have been documented till date, only about 71 [25] to 80 [26] fungal species have been known to exhibit bioluminescence. All of the said species have been unequally classified into four distinct lineages that are not so closely related [23]. "Honey Mushrooms" of the Armillaria lineage, the causative species for foxfire phenomenon, and the "Jack-o-Lantern Mushrooms" from the Omphalotus lineage are common examples of bioluminescent fungi. The origin of fungal bioluminescence can be attributed to a single evolutionary ancestry, the proof of which has been given by cross-reactions between the luciferins and luciferases of distant lineages to yield light successfully [21].

The purpose behind the emergence of fungal bioluminescence still remains elusive. Speculations have been made by Oliveira et al. that it may serve as a mode of attraction for insects, facilitating entomophilous spore dispersal, as seen in some species of *Neonothopanus* [27]. Furthermore, the same study revealed that there is some semblance of circadian control to make this entire affair more energy efficient by increasing bioluminescence at night. However, this is not true for all fungal species, wherein this trait may simply be a luminous by-product of metabolism, without a definite purpose [28]. The evolutionary feasibility of such cases is yet to be determined.

3.3 Bioluminescence in protists

Among protists, the chief groups that exhibit bioluminescence are Radiolaria (or Radiozoa), and Dinoflagellates, which are both exclusively marine. Both of these are described as follows:

3.3.1 Bioluminescent radiolaria

Among all the radiozoa, only two genera, namely *Collozoum* and *Thalassicola* are known to exhibit bioluminescence. Both of these belong to the order Collodaria, and use coelenterazine as substrate [4].

Bioluminescence has also been reported in some other deep sea species like *Aulosphaera* spp. and *Tuscaridium cygneum* [4].

3.3.2 Bioluminescence in dinoflagellates

Dinoflagellates are a group of cosmopolitan protistan organisms [29] having an ancient evolutionary history, which form one of the most important groups of phytoplankton in the aquatic ecosystems [30]. They are the only photosynthetic organisms that are capable of bioluminescence [30], and are the most dominant contributors to the occurrence of this phenomenon in the upper ocean [31]. Common phenomena like the "Red Tides" and the bioluminescent bays of Jamaica are because of the dramatic increase in the population of *Gonyaulax* and other dinoflagellate species. *Gonyaulax polyedra* is supposedly the most studied dinoflagellate species [20]. Other common bioluminescent genera are *Ceratium*, *Protoperidinium*, *Pyrocystis*, *Noctiluca*, [31] and *Alexandrianum* [29]. There have been inaccurate records of bioluminescent dinoflagellate species in the past, because of the presence

of both bioluminescent as well as non-bioluminescent strains belonging to the same species. Difference in the ability has been observed even between cells of the same strain [31].

The chemical structure of dinoflagellate luciferin (sourced from *Pyrocystis lunula*) is remarkably unique [20], similar only to that found in euphausiids (krill). This perhaps is an example of dietary linkage, as krill are known to source their luciferin from the food they consume [4]. Dinoflagellate luciferin is believed to be a derivative of chlorophyll [20]. Unlike most species that are autotrophic in nature, some heterotrophic species even supplement their luciferin synthesis with chlorophyll-rich diets [4].

Dinoflagellates produce bioluminescence with the help of specialized cell organelles called "scintillons", which enable them to glow only in response to shear or physical disturbance/turbulence in the surrounding water [31]. This glow is not persistent, but occurs in brief flashes. The intensity of these flashes may be affected by various factors like exposure to prior illumination, nutritional state of the cell, or even because of a diurnal rhythm [31]. There are evidences of a circadian rhythm that is operational in dinoflagellates, and also photoinhibition of bioluminescence during daytime [29]. The synthesis and destruction of luciferin is not the only method of regulation though; cellular redistribution of luciferin has been reported to be affected by the said circadian rhythm [20]. The intensity of the flashes also differs from species to species. Dinoflagellates prioritize bioluminescence second only to reproduction, to an extent that there have been reports of cannibalism under nutritional stress in order to support bioluminescence [31].

As far as the ecological purpose of bioluminescence in dinoflagellates is concerned, we are still unclear as to why these organisms take such measures to sustain it. The exact ecological context of this trait still remains unclear, maybe because of a lack of *in-situ* studies [29]. Some studies show that the flashes of light have a startling effect on copepods (the prime predators of dinoflagellates), which dart away from the prey [32]. Another speculation, called the "Burglar Alarm" hypothesis, states that the brief flashes produced by the cells upon coming in contact with a grazer (for example, a copepod) in turn attracts a predator of higher trophic level, hence protecting the cell from its own predator. This hypothesis is widely accepted, although there are no sufficient evidences of the same [4]. Furthermore, this hypothesis does not point out any clear advantage to the dinoflagellate [31].

To conclude, bioluminescence in dinoflagellates seems to be a useful but unnecessary evolutionary trait, as an accurate ecological context is yet to be determined [30]. In order to gain more knowledge on the same, coastal blooms can be harnessed as natural laboratories to study dinoflagellate bioluminescence in further detail [29].

4. Bioluminescence in animals: distribution and ecological significance

As it is expected, the complexity of bioluminescence certainly upgrades as we proceed upwards in the tree of life. There are no plants (terrestrial or aquatic) that exhibit bioluminescence. Fungal bioluminescence is rare, and has been discussed in the previous sections. Coming to bioluminescence in animals, there is a strong agreement that the evolution of bioluminescence first occurred in the ocean, as the oceanic ecosystem offers many favorable conditions like optical homogeneity, stability of environment, large areas that are almost or completely perpetually dark and a large diversity of organisms that can engage in a variety of ecological interactions [4]. This, and the fact that both luminous as well as non-luminous prey in the ocean are rich in luciferins ensures that the emergence of bioluminescence in the

ocean must have been a comparatively easy process [4, 33, 34]. The phenomenon of bioluminescence is so significant in the oceanic ecosystem, that it serves as the predominant source of illumination in many parts of the ocean [35]. Furthermore, courtships involving bioluminescence have been reported to show higher species accumulation rates than those without bioluminescence [36]. The presence of many independent coelenterazine-mediated bioluminescent systems, nine different phyla to be exact [10], indicates dietary linkage, as coelenterazine is procured by most species mainly through their diet [16]. Bioluminescence is encountered most commonly in the topmost 1 kilometer layer of the ocean, and is doubtlessly the most efficient mode of communication in the oceanic ecosystem [35]. The ability to glow is strongly habitat dependent because of various selection forces described earlier, and it is observed that there is a marked difference in the occurrence of this trait as we go deeper in the ocean [35].

Bioluminescence is also common in the terrestrial ecosystems, though it is nowhere as abundant as in the ocean. Various worms and arthropods are known to exhibit complex behaviors related to this phenomenon. It is clear that bioluminescence has a powerful impact on behavioral and ecosystem dynamics [4].

In this section, bioluminescence has been followed as a trait through various animal phyla, both terrestrial and aquatic, and its ecological significance is simultaneously discussed.

4.1 Bioluminescence in ctenophores

Comb jellies are the phylogenetically the most basic examples of bioluminescence in animals. Many species like *Mnemiopsis* [20, 37] use calcium activated coelenterazine as their bioluminescent substrate [4]. Some species, for example *Beroe forskalii* are known to produce myriad, cascading wave-patterns of intrinsic glow on their bodies, and some even emit a haze of glowing particles to startle the predator as a defensive measure, coupled with an escape response [38]. A majority of pelagic species are likely to exhibit bioluminescence [35]. The photo-proteins involved in bioluminescence in various genera like *Mnemiopsis* and *Beroe* have been studied, and are known to depend on calcium ions for their activity [39, 40].

Many comb jellies like *Pleurobrachia* and some species of the genus *Beroe* also show a startling display of rather colorful lights, in various wavelengths found in the visible spectrum. This was mistakenly believed as bioluminescence in the past. However, the said lights were not actually "produced" in the organism itself, as was evident in some studies [41, 42]. This iridescence was rather found to be a result of refraction of ambient light through the moving combs as the organism swims around [43].

4.2 Bioluminescence in cnidarians

Cnidarians in both pelagic as well as benthic zones, including corals, anemones, hydroids and medusae are known to exhibit bioluminescence. All of them use the luciferin coelenterazine as the substrate for their biochemical pathways (hence the name "coelenterazine"). Most of the pelagic siphonophores encountered show bioluminescence [4, 35]. The most common examples of bioluminescent coelenterates is the shallow-living hydrozoan Crystal Jelly (*Aequorea victoria*), the sea pansy *Renilla* and also the bamboo corals from the pelagic zone [44]. Anatomically, light producing centers, or photocytes, may be clustered or widely scattered all over the body, located around the endodermal layer [20]. The bioluminescent system of *Renilla* has been studied extensively, and attempts have been

made to triangulate and engineer the genes from the source into various eukaryotic (plant) systems [45].

Cnidarians use bioluminescence for various defensive, agressive as well as warning purposes. Some jellyfish show glowing wave patterns on their umbrellas, and even emit clouds of glowing particles as a part of their escape response [4]. Siphonophores use bioluminescence to attract prey within reach of their cnidocytes. Some jellyfish are also known to show aposematic glow, which is indicative of distastefulness. Cnidarians can gain a lot from aposematic bioluminescence, as it would not only warn the predators of the unpalatability of the individual, but also protect them from any physical injuries [4]. However, many predator species like leatherback turtles use this to their advantage, and easily locate prey like jellyfish.

4.3 Bioluminescence in annelids

Bioluminescence in annelids has independently emerged in several lineages [46], resulting in a rich taxonomic diversity [36] spanning across 45 different genera in 13 lineages of clitellates and polychaetes [7]. They are found in diverse terrestrial and aquatic habitats all across the globe.

Clitellates are the only terrestrial annelids known, including potworms and earthworms from families Lumbridae [47] and Megascolidae [48]. Most of them emit brief flashes, and secrete a slimy coelomic fluid packed with bioluminescent granules [47, 49] under mechanical, chemical [50] or electrical stimulation. The same trend is seen in benthic species from the family Chaetopteridae [46, 51]. This is basically a form of aposematism or advertisement of distastefulness or toxicity [52], due to which predator species avoid such individuals from a distance [7].

In the marine ecosystems, polychaetes are the predominant annelid species in both pelagic as well as benthic zones [53]. Unlike their terrestrial counterparts, marine annelids show an interesting diversity of adaptations of bioluminescence, which they use for a variety of functions. The swarming behaviors of *Chaetopterus* and *Odontosyllis* spp. [51] and their flashing patterns [54] have been studied in detail. The bioluminescent "bombs" of the deep-sea genus *Swima* are detonated upon the slightest disturbance, facilitating an almost ninja-like distraction while the animal swims to safety [55]. Several members of the family Tomopteridae are known to produce golden yellow light, which is quite rare in aquatic ecosystems [56]. Scale worms (family Polynoidae) emanate flashes when disturbed, and even break off one or more bioluminescent scales or even whole parts of the body [57] as decoys or sacrificial lures for the predator while they flee [46]. Some species even shoot sticky glowing mucus at the predators to hamper their mobility, distracting them while making them even more conspicuous [58]. Arrow worms (Chaetognatha) are also known to adapt similar defensive measures. Light production also wards of symbiotic bacteria that overcrowd the tubules of some annelids [59]. Bioluminescence is also used as a mode of intraspecific communication in annelids [7]. Some members of the families Syllidae and Cirratulidae exhibit bioluminescence as a part of their mating behaviors. Elaborate bioluminescent courtship displays of the genus *Odontosyllis* are even known to align with lunar cycles [52, 60].

4.4 Bioluminescence in molluscs

Bioluminescence in molluscs is represented by many unusual taxa, for example the bivalve *Pholas*, the biochemical machinery of which has been extensively studied. Also, the sea-firefly *Cypridina* is a specimen of significance, as its bioluminescent system was among the first to be studied and analysed in detail [3, 61]. The only bioluminescent organism from freshwater ecosystem, the snail *Latia*

neritoides, is also a mollusc [62]. Also, the terrestrial snail *Dyakia striata* is another bioluminescent organism that has been studied in great detail [63, 64]. Also, the snail *Hinea brasiliana* uses flashes of blue light as an aposematic signal to ward off predators [65].

Cephalopods are the prominent representatives of bioluminescent molluscs, and some of these may have been the source behind the fables of the mythical Kraken. Among squids alone, there are about 70 bioluminescent genera, both symbiotic and intrinsic [66]. Most luminescent cephalopods use coelenterazine as substrate for bioluminescence [67]. Squids are almost flamboyant in their exhibition of bioluminescence. *Euprymna* is known to be symbiotic with the bioluminescent bacteria *Vibrio fischerii* to form exclusive light organs [10] which it uses for counter illumination [68]. The vampire squid *Vampyroteuthis* has light organs all over its body, and it even shoots glowing particles from the tips of its tentacles. The squid *Taningia danae* has light organs on the tips of its arms, which it uses for intraspecific communication as well as to lure, stun and baffle prey [69]. Even some octopods are known to use bioluminescence to lure prey into their glowing suckers [4]. Cephalopods are also known to autotomize entire glowing arms as decoys if threatened. Some species of octopus also use bioluminescence in courtship displays.

An interesting fact about sperm whales is that they hunt squid by triggering the burglar alarm mechanism around themselves to attract unsuspecting squids.

4.5 Bioluminescence in insects

Insects are the most predominant terrestrial organisms that exhibit the phenomenon of bioluminescence. A majority of the bioluminescent insects are beetles (Coleoptera), click beetles (Elateridae), glowworms & railroad worms (Phengodidae), and fireflies (Lampyridae) [70]. The biochemical mechanism of luminescence is similar in all of these [71], even though each of them emit a diverse palette of wavelengths [20]. Other insects like lantern flies (Homoptera), springtails (Collembola), etc. also show bioluminescence.

Among springtails, only two families exhibit bioluminescence upon mechanical stimulation. Bioluminescence occurs only during sexual phases, and is crucial for sperm transfer. Lantern flies, for example *Fulgora lanternaria*, emit bright white light when both the sexes fly together [72]. Glowworms and Fungus gnats from the order Diptera show bioluminescence only in the larval stages, where they use their glow to attract prey and snare them in webs [73]. The larvae of *Arachnocampa luminosa* are a prime example of such behavior [74]. Female glowworm pupae also glow to attract males [72].

Click beetles show bioluminescence in all stages of life [75]. In the larval stage, bioluminescence serves as a tool to attract prey, as well as for defense. The pupae also glow when illuminated, and adults use bioluminescence for various functions like defense, mating communication and even general illumination [72]. In glowworms, on the other hand, bioluminescence is only secondary to pheromone-mediated communication. Males are rarely bioluminescent, only in the sexual stages for seductive purposes, whereas larvae and females are very luminescent. The railroad worm *Phrixothrix* is highly aposematic, as its body is lined with bright green glowing patches, while it has red headlights, which is very rare among all animals [70].

Fireflies are among the most studied bioluminescent systems, especially the north American *Photinus pyralis* [76]. All life stages in fireflies are luminescent, and firefly larvae are known to use their glow for defensive purposes [73, 77]. Illumination patterns of fireflies may differ even for different individuals of the same species, and are highly encodable [72, 77]. Fireflies have specialized organs

called lanterns in their abdominal segments, which can be controlled by the nervous system [20]. Since bioluminescence in fireflies forms the basis of various complex interspecific as well as intraspecific interactions, visual sensitivity according to the environment, time of activity and other parameters has evolved in parallel [78]. The signaling systems in firefly species are highly encodable, species specific, and crucially timed for maximum efficiency. Synchronous flashes are seen in various species, sometimes in swarms spanning 30 meters [72], producing spectacular displays like the ones at Chaophraya river, Bangkok. The biological significance of such displays are still not understood [73]. Due to the uniqueness of the signaling mechanism, some species have evolved to mimic other species specific signals. For example, female fireflies of the genus *Photuris* mimic the female signal of *Photinus macdermotti* to attract and prey upon their males [72]. Fireflies are also highly distasteful to predators, which is exhibited by their aposematic signals, a necessary counter measure to compensate for their high conspicuousness. Today, fireflies are adversely affected by the growing numbers of artificial lighting systems, which hamper their signaling and even cause direct mortality in some cases [79].

4.6 Bioluminescence in crustaceans

The evolutionary pathway of crustaceans reveals that bioluminescence has emerged multiple times. Many krill (euphausiids) are bioluminescent, showing biochemical pathways similar to diatoms [4]. Sergestids use bioluminescence for counter-illumination purposes. Cypridinids are known to release puffs of bioluminescent particles, and also have elaborate mating behaviors involving bioluminescence [4].

4.7 Bioluminescence in other Arthropods

Few luminous species of centipedes (Chilopoda) and millipedes (Diplopoda eg. *Motyxia*) have also been shown to exhibit bioluminescence [50]. Millipedes are also known to show aposematic signaling as a warning for toxicity [80].

4.8 Bioluminescence in echinoderms

Four out of the five classes of echinoderms, namely Ophiuroidea (brittle stars), Asteroidea (starfishes), Holothuroidea (sea cucumbers) and Crinoidea (sea lilies) are bioluminescent [50]. Echinoderms mostly use coelenterazine dependent bioluminescent systems, although some of them also use a novel photoprotein [4]. Bioluminescence is more commonly exhibited by echinoderms inhabiting deep seas. Many new bioluminescent taxa are still being discovered, and 70 ophiuroid species have been recognized to exhibit bioluminescence till date [81, 82].

4.9 Bioluminescence in tunicates

Many species of tunicates are known to exhibit bioluminescence, though planktonic tunicates are not as frequent exhibitors of the trait as planktonic larvacean Appendicularia. However, it cannot be ascertained accurately because some filter feeders (like *Pyrosoma*) may ingest and trap luminescent microbes and appear to be bioluminescent [50]. Species like *Balanoglossus* (Acorn worms) and *Ptychodera* of the class Enteropneusta are also known to be bioluminescent. Also, the sessile adult *Clavelina miniata* glows green when stimulated.

4.10 Bioluminescence in fish

Among vertebrates, fish are the only taxa that have the ability of bioluminescence. This trait is found in fish inhabiting all the depths of the ocean, but is most frequently encountered in specimens from the deepest recesses of the ocean [6]. Bioluminescence is found in about 1500 species of marine bony fish spanning 43 families in 11 different orders [4, 5, 83], out of which some like the anglerfish, flashlight-fish (*Photoblepharon*) and pony-fish (*Leiognathus*) harbor symbiotic bacteria in discrete, specialized light organs, while others produce glow intrinsically [84]. On the other hand, only a handful of shark species in three families of cartilaginous fish are known to exhibit bioluminescence [83]. Unlike bony fish species, cartilaginous fishes do not rely on symbionts for bioluminescence [85], but use an altogether different, unknown bioluminescent system [86]. Some other species like the midshipman fish *Porichthys* and various lantern-fish obtain their respective luciferins from dietary sources [13].

Fish use the ability of bioluminescence for a variety of applications like communication, evading predators, luring prey. The latter is highly expressed in various taxa inhabiting the deep seas. Various anatomical modifications (like the light organs in various bony fish and the esca of anglerfish) harbor symbiotic bacteria, which enable the fish to use the bacterial emission with ample control on the intensity as well as distribution of the emission [4]. Fish of the order Stomiiformes (like dragon-fish, etc.) have evolved most elaborately arranged photophores, including those emitting red light [4]. Cookie-cutter sharks are interesting examples of both counterillumination and mimicry, as they bait their prey with non-luminescent patches on their bodies that look like small fish.

Bioluminescence may also prove disadvantageous to some species in certain cases. For example, elephant seals follow bioluminescence to track down prey populations. Some studies have shown that seals prefer to hunt in locations where there are more bioluminescent individuals [4].

5. Future prospects

Even though we still need to understand the dynamics and biochemistries of many bioluminescent systems in nature, humans have already begun to put bioluminescence to various applications. Bioluminescent mechanisms have been used in the diagnosis of various pathological conditions in the form of Green Fluorescent Proteins (GFP) [20]. Furthermore, attempts are being made to incorporate bioluminescent systems into plants to supplement illumination [87–89]. However, these prospects are still in their developmental stages, and there are various challenges and issues that need to be tackled.

6. Conclusion

The emergence of bioluminescence in nature has occurred independently on multiple occasions, which certainly means that it confers some significant evolutionary advantage(s) which we are yet to understand fully. This is bolstered by the fact that there are so many species that exhibit this trait, and show a plethora of behavioral, anatomical and ecological trends so as to survive and thrive in various habitats. With a better understanding of these systems and their interactions, we will certainly be able to use this phenomenon to our advantage. However, there are some challenges that keep us from fully exploring certain bioluminescent systems.

For example, the deep sea bioluminescent systems are very hard to access, and thus *in-situ* observations are few and far between. With the advent of new tools and techniques, we shall be able to gain a better insight into the dynamics of these systems.

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Author details

Aditya Srivastava and Kalpna Katiyar*
Department of Biotechnology, Dr. Ambedkar Institute of Technology for Handicapped, Kanpur, India

*Address all correspondence to: kalpna@aith.ac.in

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