PUPAL EMERGENCE IN BUTTERFLY CONSERVATORIES: A SURVEY OF CONDITIONS AND PRACTICES

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Emergence of pupae is a question of shared concern for both suppliers and exhibitors, both for economic reasons and as a simple question of animal welfare. As producers and exporters of butterflies, we at Heliconius Butterfly Works have observed widely varying results in emergence rates for our pupae in different exhibits, a phenomenon noted by other producers as well.

How well butterflies emerge depends on a host of factors, many of which are beyond the control of the exhibitor. These include apparently innate differences in emergence rates for different species, problems originating in breeding (disease, inadequate handling, parasitism, inbreeding) and in transport. Nevertheless, how pupae are treated in conservatories undoubtedly also affects emergence and quality.

To try to get a sense of how conditions and practices vary amongst different exhibitors, we sent out a questionnaire to over 100 permanent and temporary exhibits around the world. We received responses from 28 facilities, detailing kinds of emergence cage, environmental conditions, techniques for hanging pupae, and other practices. We summarize the results here, in hopes of contributing to a discussion within the industry about best practices to ensure optimum results for pupae and butterflies. Unless otherwise noted, percentage results are based on a total of 28 responses.

Emergence Cases

Every exhibit seems to have developed a slightly different type of emergence case. By far the most common material reported for emergence cases was wood (usually combined with glass, plexiglass or mesh), used by at least 15 respondents. Nine exhibits used cases made of other materials, including aluminum, plastic, styrofoam, stainless steel or glass. Remaining responses were unclear as to the primary material used. Despite the fact that most facilities use wood for their cases, several expressed dissatisfaction with this material since it is difficult to sterilize.

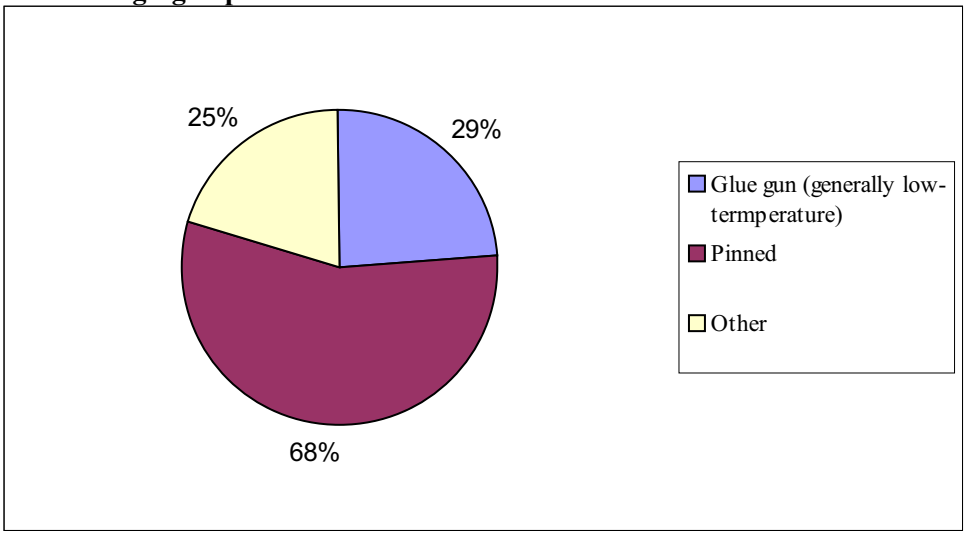
Size of emergence cases varied enormously, from walk-in rooms down to cases or boxes as small as 30 x 20 x 10 cm. As a result a very broad range was reported for numbers of pupae in each emergence case, from 500 up to 2700. Over two-thirds of exhibitors (68%) reported under 500 pupae per case. Mark Deering of Missouri Botanical Garden noted: "There are some few species that are better emerged in smaller more secluded cases than a large emergence box. These include, *Mechanitis*, *Tithorea*, *Idea*, *Euploea* and other danaids, *Heliconius doris*, *Pachliopta aristolochia* sp.. The reasons vary, some like more time to dry with no disturbance (*Idea*, *Pachliopta*) some like higher humidity (*H. doris*, *Mechanitis*)."

Although the question was not asked specifically, most exhibits maintain the emergence area in, or visible from, the main exhibit area, while others keep pupae "off-site" in laboratory or quarantine room.

Hanging Pupae

Techniques and materials for hanging pupae vary between facilities, and within facilities depending on the type or condition of the pupa. Pupae for the majority of pupae are hung from a a variety of supports, including wooden dowels, plastic rods, foam tubes or cork. This latter material was again questioned by several respondents difficulties in keeping it clean and/or sterile.

Figure 1: Methods used for Hanging Pupae



Other methods or materials reported were silicon, clips, rubber foam holes, other adhesives (Copydex, Evostick, aquarium sealant) and hooking the pupa into old silk or cotton wool. Results above add up to more than 100% because most exhibitors reported using more than one method depending on the condition of the pupa. Pinning was the preferred method, with many respondents reporting alternatives when silk was absent. Several exhibitors emphasized the use of low-temperature glue guns, as opposed to the hot-melt variety, which could damage pupae.

Sterilization

Sterilization frequency varied between facilities. One respondent highlighted the difference "cleaning" and "sterilizing", and unfortunately due to the wording of the questionnaire it was not clear that all responding to the questionnaire were using terms in the same way. Unfortunately we also missed the opportunity to ask how people sterilizebleach, heat, steam, or other disinfectants.

Table 1: Frequency of Sterilization of Pupal Emergence Case

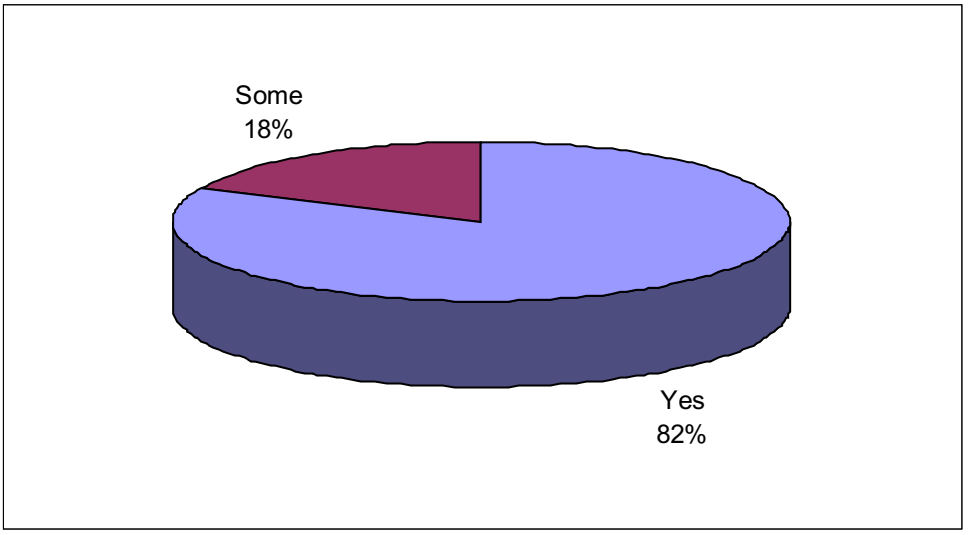
Frequency	Percentage of respondents
Between each new set of pupae	29%
Ocassionally (no specified frequency)	25%
Daily	7%
At least once a week	25%
Monthly	11%
Every 2-6 months	7%

Note: Results add up to more than 100% due to rounding and because one exhibitor sterilizes both between each set of pupae and every 3-4 days.

Recognizing diseased pupae

Diseases and parasitized pupae represent not only direct losses for exhibitors and suppliers, but also the possibility of introducing pests and pathogens. These may affect other pupae and butterflies in the exhibit, and in a worst-case scenario represent a risk of introducing potentially damaging organisms into the broader environment.

Figure 2: Staff training in recognizing diseased pupae



While the majority of respondents reported that staff were trained to recognize and remove "problem" pupae, a couple of respondents noted that this is an area that surely needs improvement.

Meconium dripping

At the November, 2003, ICBES meeting in Venice, Italy, meconium dripping onto pupae was mentioned as a possible factor affecting emergence. Twenty respondents (71%) responded that the way pupae were hung did not allow meconium to drip on other pupae, while six (21%) indicated that it did, and the remaining two (7%) noted that this could occur "occasionally." No comments were received as to whether this might be a factor affecting emergence.

Removal of dead pupae and exuviae:

The accumulation of dead pupae and exuviae (cast pupal shells) could be a factor, in theory, for transmission of disease or propagation of parasites. Frequent and opportune removal should limit this risk.

Table 2: Frequency with which dead pupae and exuviae are removed

Frequency	Percentage of respondents
Daily	54%
Between hanging each new set of pupae	25%
Every few days	25%

Note: Results add up to >100% because one respondent reported cleaning out dead pupae and exuviae daily and between hanging each new set of pupae.

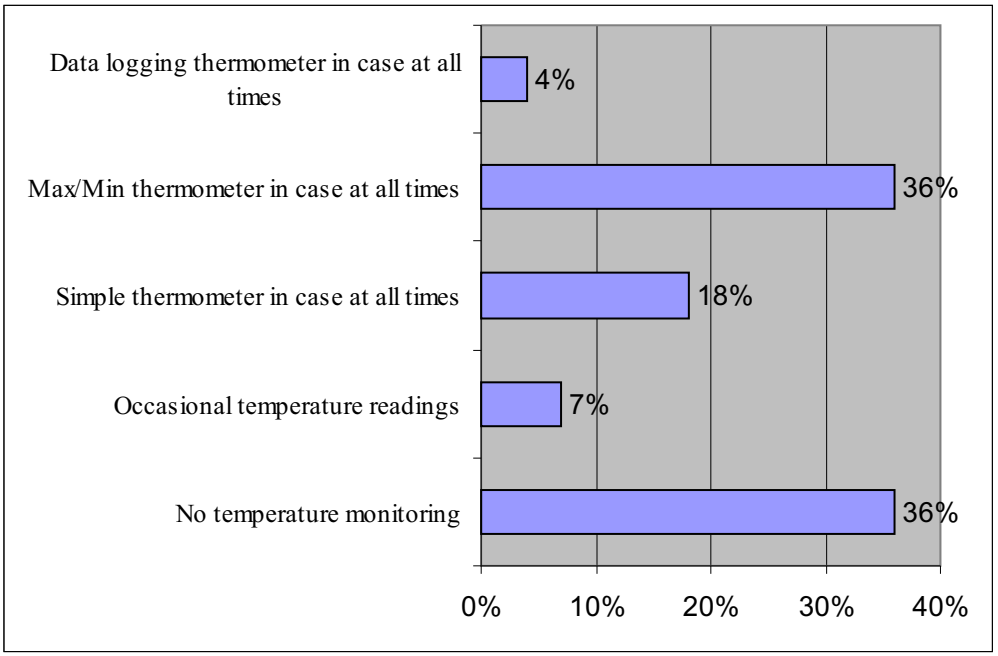
Environmental Factors

Butterfly species flown at conservatories come from a broad range of temperate, subtropical and tropical environments, and have evolved to adapt to specific natural temperature and humidity regimes. Undoubtedly, extremes of temperature can affect pupal emergence times and quality, as can extremes in humidity. I have also heard day length and even atmospheric pressure suggested as factors influencing emergence.

Temperature

Temperature can be a critical factor in pupal emergence. Excessive heat or cold can kill or deform pupae, and temperature influences the length of the pupal stage. Temperatures to which pupae are exposed are sometimes monitored:

Figure 3: Temperature Monitoring Methods



Only a minority of facilities had temperature controls specifically for the pupal emergence case: nine out of twenty-eight respondents (32%). This number includes situations where the emergence/quarantine room is temperature controlled. The remainder had no specific temperature controls, with seven respondents (25%) highlighting that temperature was dependent on the temperature regulated for the flight or exhibit areas where the case was located.

For those facilities where data is available, temperatures ranged significantly. Approximately half of all exhibits reported significant seasonal variation in temperatures.

Table 3: Maximum, Minimum and Average Temperatures Reported for Pupal Emergence Area

Max Temp C		Min Temp C		Average Temp C			
Temp Range C	**% Respondents**	**Temp Range C**	**% Respondents**	**Temp Range C**	**% Respondents**		
27-30 C	50%	14-17 C	22%	21-23 C	20%		
32-35 C	33%	18-22 C	56%	25-27 C	40%		
38-45 C	17%	25-29 C	22%	28-30 C	40%		

Note: Maximum and minimum temperatures of a total of 18 responses; average temperatures out of 15 responses

Humidity

There is a general consensus that humidity is a second critical factor determining emergence success, with a variety of techniques used to avoid excessively dry conditions.

Table 4: Methods for Regulating Humidity of Pupal Emergence Area

Method	Percentage of respondents
Damp medium in case	57%
Manual misting[1]	54%
Automatic humidifier	7%
Humidifier turned on manually as needed	7%
Greenhouse humidity regulated[2]	21%
None	11%

Notes:[1] Often in combination with other methods, only 7 respo
Ndents reported this as their sole method.
[2] Greenhouse humidity regulated very precisely in some cases, less so in other. Includes automated systems, timer-based misting and manual hosing of surrounding areas.

By and large, monitoring of humidity received less attention than temperature.

Figure 4: Methods for monitoring humidity in pupal emergence areas

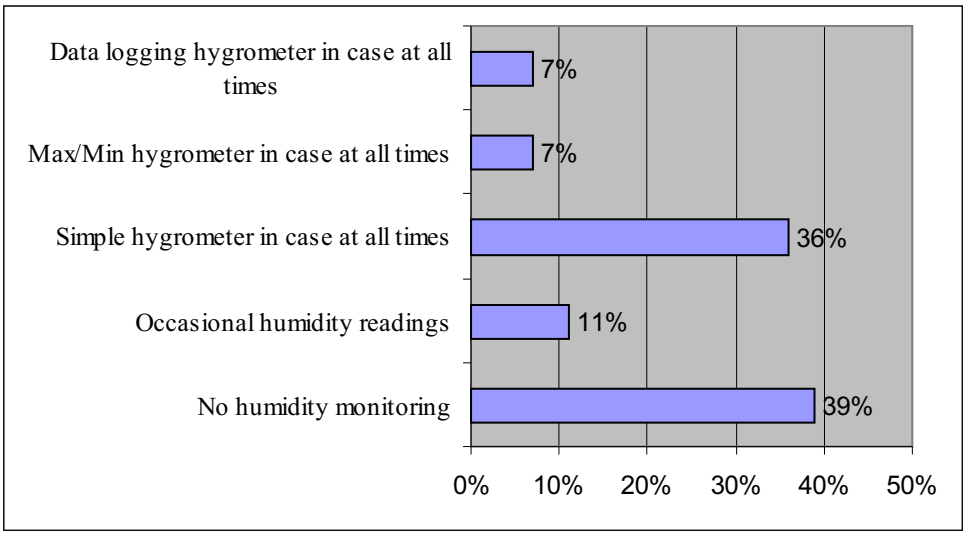


Table 5: Maximum, Minimum and Average Relative Humidity Reported for Pupal Emergence Area

Max Relative Humidity %		Min Relative Humidity %		Average Relative Humidity %			
RH Range	% Respondents	RH Range	% Respondents	R H Range	% Respondents		
54%	7.7%	33-45%	38.5%	49-60%	18.2%		
75-85%	53.8%	50-60%	46.2%	65-75%	63.6%		
89-93%	39.5%	70%	15.4%	80-85%	18.2%		

Note: Maximum and minimum humidity of a total of 13 responses; average humidity out of 11 responses

Sunlight and Ventilation

Direct sunlight can create spikes in temperatures and increase the risk of drying out in pupal cases. Most exhibitors appear to have taken into this account, with 21 of 28 respondents (75%) indicating that pupal cases were kept out of direct sunlight. Six of 28 respondents (25%) reported that pupae were exposed to direct sunlight at some time of day.

Aeration or ventilation of pupal emergence cases can likewise influence temperature and humidity. Inadequate ventilation may increase the risk of damage to pupae from fungi and bacteria.

Table 6: Method for Ventilation/Aeration of Pupal Emergence Case

Method	Percentage of respondents
Holes, mesh or opening case	53.6%
Fans or blower	39.3%
Closed case	7.1%

Other comments

Very few questionnaires were returned with emergence information, making meaningful comparisons and analysis difficult.

Roger and Sabine Gass, of the Butterfly and Orchid Garden in Thames, New Zealand noted:

> "Our emergence rate was variable with losses up to 40% to 50%, although the quality of pupae was very good. Paticularly with birdwings and heliconia butterflies. Symptoms were: normal looks at arrival then slow development or late emergence, weakness at emergence, incomplete emergence or death after emergence or inability of flight.
> My theory is that in addition to the low temperatures at transport, the increase of CO2 in the transport case leads to symptoms of poisoning.
>
> We asked our main supplier to poke tiny holes with a pin into the transport box, if 2 boxes in both of them. Therefore CO2, which is heavier than normal air will transfuse. Since we started this new procedure, our emergence rate has risen to the usual 85 to 90%. It is still a bit early to make final conclusions, but we think that it might be a solution for an old problem especially for long transit times like to NZ."

Closing thoughts

This survey has sought to describe practices and conditions found in butterfly exhibits around the world. Correlating practices and conditions with emergence success would be ideal, but is beyond the scope of this paper and impossible with information from this survey. Nevertheless, I hope that the results here presented will serve to stimulate discussion within and amongst butterfly exhibitors and suppliers as we all seek to improve performance.

In closing, I would like to highlight some points for discussion and further work:

- **Sterilization**: Cleaning and sterilization protocols are applied with varying degrees of rigor. To what extent can pathogens be transmitted within pupal emergence cases? Can fungal, protozoan or bacterial diseases contaminate the emergence case and/or neighboring pupae? To what extent do inadequately sterilized emergence cases compromise pupal emergence success or the viability of butterflies?

- **Recognizing diseased and parasitized pupae:** Better information and training is needed industry-wide to help personnel working with pupae to recognize disease problems early.

- **Temperature and humidity control:** Temperature and humidity controls are rudimentary at most facilities. Although automated systems may be beyond the means of many exhibitors, there would appear to be room for improvement in monitoring these two factors. Including max/min thermometers and hygrometers in the emergence cases is a low-cost way to record occasional spikes that may affect pupal emergence.

- **Correlating environmental factors and emergence:** Optimum temperature, humidity, space, and light conditions almost surely vary between species. Few exhibitors will be able to tailor these conditions for each species. But at the moment, little or no information is available regarding the effects of these environmental factors and pupal emergence and viability. This is clearly an area for shared research between exhibitors and suppliers.

- **What goes on in transit?** While suppliers and exhibitors make every effort to ensure the best possible conditions for pupae, what happens between point A and point B remains a great unknown. Couriers are likely to expose boxes of pupae to a range of extreme conditions, from a broiling warehouse in Panama to freezing tarmac in Chicago. This is another area ripe for collaboration between suppliers, exhibitors and our beloved couriers.

Acknowledgements

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