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Regulation and ecological importance of extended diapause in forest insects, with specific reference to the pine processionary moth *Thaumetopoea pityocampa*

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Summary

Diapause, most of the time, plays a key role in the life history of many insect species. As an alternative, or sometimes in addition, to escape in space through flight, diapause let insects skip the environmental troubles in time. Prolonged diapause is the extension of reproductive effort over several reproductive seasons or years. This feature is usually important from the anthropogenic view when any insect pest is hard to fight with. Pine processionary moth (*Thaumetopoea pityocampa*) is such a pest, having not only economic but also health impact on humans, which has adapted the trait of extended diapause. All efforts in making prediction of trend of population dynamics as well as eradicating this pest in the forests went in vain due probably to this trait. The issue of prolonged diapause should be well understood if we want to put a stop into the negative impact of this obnoxious species. Considering the problem, we have made a preliminary effort of understanding the ecology and regulation mechanism of this species in Venosta valley, the Southern Alpine region of South Tyrol in Italy. It is a range expansion area of pine processionary where a sudden outbreak occurred in 1998 and 1999. Local forest department sprayed *Bacillus thuringiensis kurstaki* (Btk) over infested area from then on to suppress the population. Our main objectives of the study were to assess the timing and size pattern of normal diapausing and extended diapausing individuals. In addition to these, we tried to understand the intensity of prolonged diapause, population dynamics over years, and the impact of temperature and rainfall in the timing of first emergence each year. Historical data of rearing cage experiment and catch in pheromone traps were collected from the forest department with which analyses were made. We found at least on average 27 days of gap between the emergence of diapausing and non-diapausing individuals where diapausing individuals always came out in advance. Significant different in the size of extended

diapausing and non-diapausing individuals has initially been found. Cage experiment resulted at least seven years of prolonged diapause in the Venosta population. Percentage of extended diapausing individuals has been found to be very high. Apart from this, population dynamics graph showed clear fall in the number of population following insecticide spray and establishment of natural enemy. We initially found that temperature and rainfall can explain the variation in first emergence date each year very well. We recommend further study on various issues regarding extended diapause in the species, which will in turn make us understand the whole scenario of mechanisms that make it established pest. Structured study on the issue may open a new window of managing pine processionary in an eco-friendly and effective way.

1 Introduction

1.1 Background

Environmental conditions are not the same everywhere in the earth. These vary from time to time and space to space, so do various life forms flourished on earth. Insects are intriguing creature of nature. Immense number of insect species have been predominating the world since the time immemorial. Evolution has shaped them achieve this success through modifying their life history so that they cope with diverse habitats throughout the world. Searching out how insects adapt with the adversity or favor of seasonality in different habitats and what strategy they follow have been a long-standing thirst for entomologists. Let's take a cursory look on how the history of study on seasonality approached with time.

At first, temperature was assumed to be the main factor of seasonal activity and development of animals and plants. Afterwards, scientists started thinking on daylength to be the key factor of plant development. Soon after Garner and Allard (1920, 1923) invented the term photoperiod and photoperiodism, other scientists started finding the similar occurrence in other life forms including insects (see for insects: Marcovitch 1923, Sabrosky et al. 193, Kogure 1933, Müller 1960, for birds: Rowan 1926). Photoperiodism could be able to explain so many features of plants and animals that Danks (1987) wrote in his book, "Photoperiodism captured the imagination of the scientific world,....". However, physiologists afterwards discovered the role of hormone in diapause. Although, huge excitement on insect diapause over the decades of 1960s and 1970s resulted vast information on hormonal and environmental aspect of diapause, soon after, the field entered into diapause (Denlinger 2008). Subsequently, breakthroughs in molecular biology widened scope for discussing evolutionary and genetic aspect of diapause, along with evolution of life cycles in insect (Danks 1987).

1.1.1 Few terms related to dormancy

Before going to further discussion, defining few terms is necessary for the clarification. Dormancy includes both diapause and quiescence from broad point of view. There were few attempts of defining terms previously of which work of Shelford (1929), Lees (1955) and Danks (1987) are worth mentioning. Thereafter, Koštál (2006) reviewed eco-physiological phases of diapause in insects where he tried to simplify the terminologies. He defined dormancy as a generic term that covers any state of adaptive suppressed development, generally accompanied with metabolic suppression. Danks (1987) compared the features of quiescence and diapause with synonyms used by other scientists. He featured quiescence to be immediate response where environmental factors directly limit development and arrested development is simultaneous with adverse condition. Synonyms of quiescence tabulated were "physical rest" (Ushatinskaya 1976), "consecutive dormancy" (Müller 1965, 1970), "torpidity" (Ushatinskaya 1957, 1976), and "pseudo-diapause" (Roubaud 1930, 1953). On the other hand, diapause was characterized as programmed (delayed) response where environmental factors indirectly modify developmental pathways and suppressed development lasts longer than adverse condition. Few alternative names for diapause provided were "physiological rest" (Ushatinskaya 1976), "spontaneous arrest" (Shelford 1929), "prospective dormancy" (Müller 1965, 1970), "auto-dormancy" (Flanders 1944), and "diapause vrai" (Roubaud 1930, 1953). However, Koštál (2006) define quiescence and diapause as follows:

Quiescence: An immediate response (without central regulation) to a decline of any limiting environmental factor(s) below the physiological thresholds with immediate resumption of the processes if the factor(s) rise above them.

Diapause: A more profound, endogenously and centrally mediated interruption that routes the developmental programme away from direct morphogenesis into an alternative diapause programme of succession of physiological events; the start of diapause usually precedes the advent of adverse conditions and the end of diapause need not coincide with the end of adversity.

1.1.2 Why study diapause

Though the study of diapause stems mainly from the curiosity to unveil the mystery of the world, scientists and technologists started find out practical implication of the knowledge (Danks 1987). Denlinger (2008) wrote an excellent entry on the potentiality of studying diapause. He argued for diapause not only to be necessary for understanding life history of insect, but also as potential tool for inventing new technologies, in addition to provision of fundamental insights into critical issue in development. The areas he pointed out in bold letter as the direct beneficiaries of diapause researches are: population modeling, manipulation of diapause for control, exploitation of diapause traits for pest management, managing domesticated species, increasing shelf-life and utility of biological control agents, cryopreservation, foundational studies of insect hormones, a fascinating question in developmental biology, links to aging research, model for obesity, tools for blocking disease transmission, and pharmacological prospecting. In addition, interesting insights on the molecular basis of *aging* can come out from the study with diapause (Denlinger 2002). Danks (1987) also exemplified few prospects of diapause research in his monograph on insect dormancy (see page 2).

1.1.3 Stages of diapause

Diapause in insects is conceived as a process rather than just a status (Tauber 1986, Danks 1987, Denlinger 2000, 2002). Yet, there has been debate among authors on phases of this process. Danks (1987) showed various stages of diapause in his monograph. He pointed out two main problems hindering the attempts of defining terms. First, there is no obvious marker indicating gradual internal changes. Second, attempts to subdivide phenomena that are continuous. However, former problem is now solved since the gap of knowledge is filled by the molecular or physiological study and later problem is nothing but a general methodological one (Koštál 2006).

Table 1: Stages of diapause with synonyms (adapted from Danks 1987 and Koštál 2006)

Stage and indicator	Synonyms
1. Pre-diapause	
1.1 Preparation (storage of information about developmental destiny, different behavioral activities or physiological process, food storage, some changes in development rate etc.)	Preparation phase (Mansingh 1971) Prediapause preiod (Tauber et al. 1986)
1.2 Induction [sensitive period] (diapause programmed endogenously (for obligatory) or by exogenously (cues or token stimuli))	Part of diapause induction period (Tauber et al. 1986)
2. Diapause	
2.1 Initiation [responsive stage] (direct development ceases; regulated metabolic suppression; mobile diapause stages may continue accepting food; building energy reserves and seeking suitable microhabitat; probable physiological preparation for adverse period and increased intensity of diapause)	Entry (Danks 1987) Onset, beginning, start; part of diapause induction period, and diapause intensification (Tauber et al. 1986)
2.2 Maintenance (persistence of developmental arrest though having favorable condition for direct development; specific token stimuli may help maintain diapause; relatively low and constant metabolic rate; gradual decrease of diapause	Diapause development (Andrewartha 1952, Lees 1955, Tauber and Tauber 1976, Danks 1987) Physiogenesis (Andrewartha 1952, Tauber and Tauber 1973) Conditioning (Morden and Waldbauer 1980, Waldbauer 1978, Waldbauer and Stenburg

intensity and increase of sensitivity to diapause terminating condition due to unknown physiological process(es).	1978) Restoration period (Ushatinskaya 1976) Refractory period (Mansingh 1971) Endogenous phase (Dobrinojević 1980) Acquisition of competence (Williams 1956) Maturation (Agrell 1951) Diapause process (Zolotarev 1947) Diapause ending process (Schneiderman and Horwitz 1958) Latent development (Agrell 1951) Reactivation (Danilevsky 1965) etc.
2.3 Termination (competence to resume activity if conditions are permissive)	End of diapause development (Danks 1987) Completion (Hodek 1964) Quiescent phase (Dobrinojević 1980) Termination or termination phase (Tauber and Tauber 1973, Hinton 1957, Mansingh 1971, Waldbauer and Sternburg 1973) Diapause broken Post-diapause transitional period-quiescence (Tauber et al. 1986)
3. Post-diapause (reorganization before full activity)	Post diapause development (Danks 1987) Morphogenesis (Andrewartha 1952, Tauber and Tauber 1973, 1976) Growth development (Andrewartha 1952) Post-diapause transitional period-morphogenesis (Tauber et al. 1986)
Completion (activity resumes)	Reactivated (Way 1962)
Non-diapause development (uninterrupted development)	Continuous development

A question might arise if there is any taxonomic or geographic effect in determining diapause stage. The answer varies. General observation shows related groups to be dormant in the same stage. But, otherwise is true as well. Moreover, many insect species have more than one diapausing stage (e.g., see Thiele 1977). Type of life cycle and species determine entry in diapause of *Schiomyzid* flies (Berg et al. 1982). However, stages are most of the time fixed within genus or species (L'Vovsky 1975) with rare exceptions. In addition, a few species can experience more than one diapause during their life cycle (Danks 1987).

For arctic species, larval stage is quite common for the diapause (Johansen 1921, Danks 1978), whereas this commonness reduces in the relatively southern latitudinal temperate species (Danks 1987).

1.1.4 Classification of diapause

Different authors have tried to classify diapause; therefore, different schemes are available. Danks (1987) tabulated various attempts of classification in his monograph. I review here very concisely few schemes of diapause classification presented in Danks (1987) with little adaptation.

Müller (1970) classified diapause based on the diapause inducing and terminating environmental condition. Classes are:

Table 2: Müller's classification of diapause

Category	Characteristics
Oligopause	Induced by suboptimum factor and terminated by same factor. Appears and ends with a delay.
Eudiapause	Induced usually by photoperiod, while ended by factors other than inducing one. Clear induction phase present.
Parapause	Obligatory; not dependent on environment for induction. Terminated by one or two alterations in one environmental factor.

Witsack's (1981) scheme was an extension of Müller's (1970) work. His classification includes:

Table 3: Witsack's classification of diapause

Category	Characteristics
Oligopause	Induced by unfavorable conditions while ends by unfavorable conditions as well. Appears and ends with a delay relative to conditions.
Eudiapause	One factor is responsible for the induction while different factor ends diapause.
Parapause	Optimal conditions initiate diapause. Unfavorable condition is responsible for the end.
Primary parapause	Unfavorable condition can prevent this type of diapause
Secondary parapause	Prevention is not possible
Hyperpause	Both induction and termination determined genetically. This diapause is obligatory.
Superpause	High intensity diapause

Mansingh (1971) developed another sort of scheme and classifies dormancy based on seasonal constraints. In relation to conditions, according to his classification, dormancy is of following types.

1) *Hibernation*: It occurs due to temperature below the optimum.
2) *Aestivation*: Temperature higher than optimum induce this dormancy.
3) *Athermopause*: It happens due to factors other than temperature.

He classifies dormancy according to the depth as well. Those are:

1) *Oligopause*: Mild and long-term adversity make this happen. Organism takes preparation right before constraint; short refractory phase is present.

2) *Diapause*: Extreme and long-term adversity causes this dormancy. Individuals take preparation long before adversity; long refractory phase present. There are two types of diapause:

2.1) *Ateleodiapause:* Low intensity diapause.
2.2) *Teleodiapause:* High-intensity diapause.

Ushatinskaya (1976) modified the approach of Mansingh (1971) and included depth of dormancy in the type Aestivation and Hibernation. The following depth has been identified in both type of dormancy:

1) *Sleep*: Daily relaxation of tension
2) *Oligopause*: Intermediate between sleep and diapause
3) *Diapause*: Deep and continuous diapause
4) *Superpause*: Diapause for more than one adverse season.

Horotelic and tachytelic process of diapause development:

Morphogenesis after diapause can be resumed through two types of process of diapause development. The processes that progress at a lower rate are dubbed as horotelic (or spontaneous), while accelerated processes are called tachytelic (or inductive) (Hodek 2002, Zaslavsky 1988).

1.1.5 Regulation of diapause

Diapause is regulated at several levels. Interaction of environment and genetic pre-programming combines with physiological process in this case. Fortunately, we have excellent entries on the environmental (Tauber et al. 1986, Danks 1987), Hormonal (Denlinger et al. 2005), and genetic (2002) regulation of diapause. In addition, Saunders et al. (2002) discussed in detail the role of photoperiod in the regulation of diapause.

Seasonality is very important for the diapause of temperate insect species. Some physical environmental factors regulating diapause are photoperiod, thermoperiod, temperature level, moisture, sunshine, and wind. Non-physical environmental cues include occurrence of other organisms, food, and metabolic state of host (Danks 1987). However, interaction among factors (Danks 1987) is common and worth taking into account. Nevertheless, photoperiod seems to be the most reliable cue for the diapause (Danks 1987, Saunders et al. 2002).

Apart from environmental regulators, several key hormones are involved in the regulation of diapause. Those important hormones are – juvenile hormone, diapause hormone, and prothoracicotropic hormone (Denlinger et al. 2005). On the other hand, though in the rudimentary level, works on the molecular regulation of diapause are producing significant insights into dynamic process of diapause, such as changes in the expression patterns throughout the development of diapause (Denlinger 2002, Yocum et al. 2011).

1.1.6 Prolonged diapause

Emergence after diapause is not always restrained in the same reproduction year. Cases are common where insects remain in the diapause for more than one adverse season. Different authors have named this phenomenon differently-prolonged diapause, extended diapause, perennial diapause, superpause, super diapause, remanance, überliegen etc. (Danks 1987, Saulich 2011). Few parasitoids may go on extended diapause in the first instar larva and wait until their host resumes development (Danks 1992). Parasitic larvae can diapause on the tissue of their diapausing host (Saulich 2011). Danks (1987) enlisted species that can remain in diapause for more than one adverse season. Saulich (2011) reported over 150 species from different taxa having extended diapause. In spite of having variability in emergence, in most cases, superpause last for around 2 to 3 years, but it may extend to more than a decade in extreme cases (Danks 1987, 1992, Hanski 1988). This prolonged diapause has been identified as one of the four mechanisms for long life cycle in insects (see review by Danks 1992 and Saulich 2011). Other three mechanisms are slow larval development, prolongation of the adult stage with repeating reproductive period and combination of all previous three mechanisms in one life cycle. An interesting case of larval development is wood feeding buprestid Buprestis aurulenta, which emerged up to 51 years after their infestation (Linsley 1943 and Smith 1962 cited in Danks 1987).

Prolonged diapause may take place in different stages of development in different order of insects. However, ability to enter superdiapause in a particular stage is fixed at the species level (Saulich 2011). Percentage of superdiapausing individuals may vary according to the severity of the climate thus different latitudes. Duration of superpause is extremely varied. It varies between different species, between populations of the same species, even between individuals within the same population. Genetic heterogeneity is obvious from the observation that the progeny of the same female may vary in the duration and intensity of the diapause (Saulich 2011).

Superdiapause is a characteristic of numerous phytophagous insects with wide fluctuations of abundance and periodical outbreaks (Danks 1992, Saulich

2011). The number of superdiapausing pronymphs of the European pine sawfly varied not only from season to season, but also in different age of planted pine stands. Older stands provided more favorable condition for superdiapausing than younger plantation (Minder 1973). Unfavorable diet (old needles or spring pine shoots) was more favorable than usual diet (previous year pine needles) for entering superdiapause (Stadnitskii 1964). Stadnitski (1971) supposed that the regulating factor would be same both for onset of the superdiapause and bud formation in the food plant. Saulich (2011) suggests from few experimental data that superdiapause should occur more frequently in the regions where larvae develops in the second half of summer with days shorter than 16 hours. This condition is common in at high latitudes and in the mountains.

1.1.6.1 Characteristics of superdiapausing individuals

There is very less difference between superdiapausing and one-year diapausing individuals. However, Intensity of respiratory metabolism during superdiapause is 10-40 times less than in the active state and 2-3 times lower than at the peak of hibernation in colorado potato beetle (Ushatinskaya 1983). Loss of weight in *Chrysopa regalis* and *Chrysopa dorsalis* after superdiapause is less than that of after hibernation (Volkovich 2007). Body mass was found greater in the pronymph of the spruce seed moth *Laspeyresia strobilella* (Nesin 1984) and older instar larvae of the fungus weevil *Exechesops leucopis* (Matsuo 2006). Degree of pigmentation of the pupal cuticle is an indicator of superdiapause in pharate adults of *Barbara colfaxiana.* Superdiapausing individuals in this case has pale cuticle (Sahota et al. 1985). Though Saulich (2011) states that females after superdiapause have similar level of fecundity as of one-year diapausing female in colorado potato beetle, Wang et al. (2006) found the female with superdiapause to be highly fecund than their regular diapausing counterparts in *Colaphellus bowringi.* However, lower fecundity and higher mortality in the individuals with extended diapause have also been reported (see for example, Sullivan and Wallace 1967, Sims 1983). Despite insufficient study conducted for extended diapause, available data advocate that, superdiapause differs from annual diapause in a deeper level of metabolism suppression as a means of increasing chance of avoiding adverse external conditions (Saulich 2011).

1.1.6.2 Regulation of prolonged diapause

Most of the species show decreasing pattern in emergence in the successive years, where most of the individuals emerge after only one winter or summer (for example, see for sawfly *Gilpinia hercyniae* (Prebble 1941), for blumaggot *Rhagoletis mendax* (Lathrop and Nickels 1932), for walnut husk fly *Rhagoletis completa* (Boyce 1931), for the gall midge *Contarinia sorghicola* (Baxendale and Teetes 1983) and for the cabbage beetle *Colaphellus bowringi* (Wang et al. 2006)). However, Barnes (1958) reported anomaly of the previous case in gall midge *Sitodiplosis mosellana.* This pattern was assumed to be due to response to environmental cues. Danks (1987) accumulated few examples supporting this phenomenon, where prolonged diapause was associated with an abnormally hot dry summer after the first year in the apple maggot *Rhagoletis pomonella* (Allen and Fluke 1933), cool summer temperature in the larch sawfly *Pristiphora erichsonii* (Drooz 1960), low temperature and high humidity in eonymphs of *Neodiprion sertifier* (Minder 1981), density in the same species (Eichhorn 1983), lower temperature in laboratory experiment but low cone crop in the following year in field experiment in cone moth *Barbara colfaxiana* (Hedlin et al. 1982), year with lower cone crop production in several species of cone and seed feeding insects (Hedlin et al. 1981), photoperiod in Neodiprion abietis (Wallace and Sullivan 1974), photoperiod and temperature in *Neodiprion sertifer* (Sullivan and Wallace 1967), and light sandy soils in *Leptinotarsa decemlineata* (Ushatinskaya 1966, 1972). Moreover, prolonged diapause may be induced in the Swaine jack pine sawfly *Neodiprion swainei* according to the depth of the soil at which the cocoons are located (Price and Tripp 1972). In addition, Volkovitch (2007) found high temperature as favorable factor for superdiapausing of lacewings in the temperate zone of Russia. Miller and Hedlin (1984) found no correlation with parental diapause habits while Ushatinskaya (1966, 1972) found heavy, moist and clay soil to be impossible for prolonged diapause in *Leptinotarsa decemlineata.* Though there might be correlation between frequency of superdiapause occurrence and the individual factors, it's almost impossible to find out factor playing key role (Saulich 2011).

Internal physiological factors may also determine the prolonged diapause in insect. Soula and Menu (2005) identified the role of initial lipid content in the prolongation of diapause in the chestnut weevil. Researching with the same species, Menu and Desouhant (2002) found, in addition to late-emerging character, bigger chestnut weevil to have increased probability of prolonged diapause. They hypothesize that variability in life cycle duration relies on the metabolic resource, such as lipid, of individual.

Apart from environmental and physiological factors discussed above, genetic component is an important regulating factor in prolonged diapause (e.g., Eichhorn 1977, Sims 1983, Tauber et al. 1986), as the onset and duration of diapause is a result of interaction between environmental cues (Danks 1987) and genetic programming (Mousseau and Roff 1987). Genetic differences control the onset or depth of many prolonged diapause (Danks 1992). In addition, conditions during induction may determine the duration of prolonged diapause in some species (for example, Sullivan and Wallace 1967 and Minder 1981). However, the duration of superpause may not be predetermined during onset of diapause (Higaki et al. 2010). Hadlington (1965) suggested moderate levels of sex linkage for the incidence of prolonged diapause. In addition, in the case of sex linkage, phenotype of a male offspring is entirely dictated by the maternal allele on X chromosome (Morgan 1909). French et al. (2012) demonstrated the heritability of extended diapause trait and strong influence of female parent in Northern corn rootworm.

1.1.6.2.1 Termination

Environmental cues, such as drought (Ingrisch 1986) or higher temperature (Miller and Ruth 1986) in the preceding summer may terminate extended diapause. In some tropical silkmoths, wetting may influence emergence from diapause (Danks 1992). Powel (1974) experimented on individuals in prolonged diapause of 33 months, where artificial watering made those individuals come out from superpause. He thus suggested sporadic rainfalls to be a terminator of prolonged diapause. Prolonged diapause can be terminated through repeated temperature cycle in several species (e.g., Neilson 1962, Higaki and Ando 2000, Higaki 2005, 2006 etc.). Intensity of prolonged diapause in chestnut weevil is dynamic and depends on the condition of ambient temperature (Higaki et al. 2010). In case of winter diapause, relatively warm temperature (such as 20-25 °C) is more effective than cold treatment in terminating prolonged diapause in several species, provided it might take longer time (2-3 years) (Higaki and Ando 2000, Higaki 2005).

1.1.6.3 Theory of extended diapause

Hanski (1988) has an excellent review on theories of extra long diapause, which comprises both premature and prolonged diapause. Since, our concern is about prolonged diapause, we will restrain ourselves in this theme.

Powell (1974) categorized prolonged diapause into two types. All individuals in the population enter prolonged diapause in one type whereas only a fraction of the individuals enters superpause in the other type. Entrance into prolonged diapause by all the individuals may occur if the resource availability or some essential environmental factors show strong multiannual cyclic component (Hanski 1988).

Prolonged diapause may be density-dependent or density-independent (Hanski 1988). Density-dependent diapause is necessarily extra long diapause (premature or prolonged diapause), while simple diapause is characteristically density-independent induced by short day-length and low temperature (Tauber et al. 1986, Danks 1987). Despite having less study, it can be assumed that density-dependent prolonged diapause may occur in some forest insects with occasional outbreaks (Hanski 1988).

1.1.6.3.1 Density-independent prolonged diapause

Cohen (1966) worked on annual plants of deserts, where rainfall is highly variable and unpredictable. Thus he assumed "risk spreading" prolonged diapause to be a better solution than simple diapause. Dispersal is an alternative to prolonged diapause. Few models predict that frequency of prolonged diapause is negatively correlated with dispersal rate. However, if the fluctuations are synchronized well across the population, it is supposed that the natural selection will favor the extra long diapause instead of dispersal (Gadgil 1971, Hanski 1988). But, this phenomenon is not tested that much in case of insects (Hanski 1988).

On the basis of list of insect species with prolonged diapause provided by Danks (1987), Hanski (1988) concluded three categories of insects with superdiapause in terms of micro- and macrohabitat occurrence. In the first category, species from the tundra, arctic and arid regions are taken into account. He assumed this occurrence of prolonged diapause to be due to the high year-to-year temporal variability than other regions like temperate one. He showed demonstration in favor of his assumption by drawing example of *Pegomya* flies breeding in *Leccinum* mushrooms, where the case of prolonged diapause has been found rarely in only one species in southern Finland (see Hackman 1976), but all seven common species of Finnish Lapland showed incidence of superpause (see Ståhls 1987). More year-to-year variability in mushroom production in the northern region than that of south region has been presumed as the probable reason for this variability in occurrence of prolonged diapause.

The second group is comprised of species who breeds in patchy and ephemeral microhabitats, such as cones, fruits, nuts, galls, mushrooms, and host individuals. Dramatic temporal variation in availability may also describe this phenomenon. According to Bulmer's (1984) model, species using more variable resource should have more occurrence of superpause than species using constant resource. Annila (1982) has experiment on *Megastigmus* supporting this view. Hanski (1988) hypothesized from the "predator satiation hypothesis" of Janzen (1971) that, prolonged diapause in insects is a counter-adaptation to seed predation that results in fluctuation of seed production in many trees. Insects must have learnt to "predict" the size of cone crop in the following year (Hanski 1988, for example, see Bakke 1963, Stadnitski et al. 1978, Annila 1981, Hedlin et al. 1982). Temperature might be one of the environmental cues in this regard (Miller and Hedlin 1984).

However, the third and largest group of insects with extended diapause, who are herbivorous, cannot be explained well by the theory of temporal variability. However, two taxa in this case are sawflies, many of which are familiar outbreak species, and butterflies, whose inclusion may be a bias as they are well studied (Hanski 1988).

1.1.6.3.2 Density-dependent prolonged diapause

Density-dependent prolonged diapause is a rare phenomenon in the nature probably due to its independence from adaptive response to most kinds of temporal variability (Hanski 1988). An interesting example of density-dependence is pine-feeding sawflies. It has abundant food resource throughout the year. In addition, occasional population outbreak cause the sizes of the population of this insect vary. Usually bivoltine *Diprion pini,* a serious outbreak sawfly, becomes univoltine during an outbreak and switches mostly to prolonged cocoon diapause at the end of outbreak (see Eichhorn 1982). According to Eichhorn (1982), an instrument of ending outbreak is the decline of population growth rate due to increasing frequency of prolonged diapause. Alternatively, prolonged diapause might be a cause of population decline. When population declines, wish to survival dominates over the wish of reproduction. Thus, prolonged diapause can avoid many negative factors (food shortage, disease, egg and larval predation, parasitism) occurring at the end of outbreak, and increase the chance of survival (for example, see Kolomiets et al. 1979). Parasitoids of pine sawflies go to prolonged diapause as well in response to the decrease in host population, however, variability in resource remains the main element of this strategy in parasitoids rather than their density-dependence.

Density-dependent extra long diapaus should be selected evolutionarily where extra long diapause happens not due to environmental variability, rather due to population dynamics. This extra long diapause helps individuals exploit the favorable breeding opportunity, which is not possible in the case of density-independent extra long diapause. Density-dependent extra long diapause may reduce population fluctuations that led to evolution of extra long diapause at first (Hanski 1988).

Apart from density-dependent and density-independent characteristics of extra long diapause, Hanski (1988) discussed about multispecies interaction in his review. Though the theoretical work is absent, he drew few examples of interspecific competition and host-parasitoid interaction and discussed on the matter.

In answering the cause of differentiated length in prolonged diapause in four dominant competing species of seed and cone insects of Norway spruce

Picea abies, all who are experiencing same level of variation is cone production, Hanski (1988) explained two reason. In the first explanation, he identified severe competition among seed and cone insects, which may restrict the number of coexisting species. There is probability of death of nearly all cones and cone-breeding insects, if the emerging population is large compared to number of cones. Second explanation says about the mutual adaptation of different prolonged diapause behavior that increases the chance of avoiding competition thus increasing probability of survival. However, polymorphism may allow a species exploiting resource regardless of temporal variability.

There is the example of simultaneous extended diapause in insects and their parasitoids. In few cases, emergence of parasitoid is well synchronized with the emergence of hosts. Annila (1981) suggests the same external cue as responsible for same timing. However, Solbrek and Widenfalk (2012) reported that, two parasitoids of gall midge *Contarinia vincetoxici* always broke their prolonged diapause in advance of breaking of superpause in their host. Nevertheless, emergence of parasitoid is sometimes belated than that of their hosts (see for example, Kolomiets 1979). Hanski (1988) threw two questions in this regard. First, whether the phenomenon of longer length of prolonged diapause in parasitoid than that of their hosts is general or adaptive. Second, whether the host-parasitoid or predator-prey dynamics only can make host and/or parasitoid enter prolonged diapause. If the density of host population reaches in equilibrium due to parasitoid, there is narrow chance for the evolution of prolonged diapause due to little temporal variation in fitness. According to Bulmer's (1984) model, however, chances should be increased if the dynamics are cyclic or chaotic. Yet, work of Corley et al. (2004) shows modest contribution of prolonged diapause in the stability and persistence of host-parasitoid interactions.

In the population level, prolonged diapause is important for the marginal and poorly adapted populations, who are prone to variable environment. In addition, when the fluctuation is entirely due to population dynamics, prolonged diapause can increase the stability of the population (Hanski 1988).

Danks (1992) recognized unpredictable habitats, unpredictable food supplies, and biotic interactions such as escaping intraspecific and interspecific competitions and natural enemies as the possible reasons of extended diapause.

1.1.6.4 Strategy of prolonged diapause

What strategy led the insects to adopt the prolonged diapause remains an interesting question. Recent studies suggest an "adaptive bet-hedging" (Philippi and Seger 1989) strategy or "coin-flipping strategy"(Walker 1986) in following variable duration in the life cycle for several species (see for chestnut weevil, Menu and Debouzie 1993, Menu 1993, Menu et al. 2000, Soula and Menu 2003, for bee *Perdita portalis,* Danforth 1999, for *Pegomiya* flies, Hanski and Ståls 1990, for *Papilio zelicaon*, Sims 1983 etc.). According to this concept of "spreading the risk" (see den Boer 1968), individual females in prolonged diapause spread the risk of their reproductive effort over more than one season that decreases the risk of elimination of entire year-class from unpredictable catastrophic events (Griffiths 1959, Sims 1983, Menu et al. 2000, Sterns 1976). From an evolutionary point of view, the long-term benefits of prolonged diapause outweigh the disadvantage of increased mortality rate (Sims 1983).

Another type of prolonged diapause is predictive or weather-dependent where diapausing individuals wait for an appropriate environmental signal representing favorable conditions. A basic difference between risk-spreading and predictive diapause lies on target individual. In the earlier case, breeding mother's fitness is affected only; none of its diapausing offspring take part in spreading the risk individually. On the other hand, predictive diapause let all diapausing individuals make their own decision and increase their own reproductive success.

A third type of prolonged diapause is disturbance-broken dormancy, although it is not present or very rare in insect. This type arises due to lack of suitable condition for reproduction (Hanski 1989).

Prolongation of life cycle into several years was assumed to be a simple extension of normal winter diapause (Tauber et al. 1986, Danks 1987, Hanski 1988, Menu 1993, Danforth 1999). However, Ushatinskaya (1984) did not provide any real evidence for her statement that led to this hypothesis. Therefore, Soula and Menu (2005) challenged the statement and presented evidence for their alternative hypothesis of "late switch" against previously assumed "early switch" hypothesis. According to "late switch" hypothesis, short and long-cycle insects (chestnut weevil) resume their development after winter diapause and long-cycle insects re-enter a second diapause afterwards.

In addition, prolonged diapause as an adaptive strategy for avoiding biotic hazards such as disease, predators, and parasites, has been demonstrated in studies with few insects (Price and Tripp 1972, Minder 1973).

1.1.7 Extended diapause in Lepidoptera

Extended diapause is present in a number of species under the order Lepidoptera. Danks (1987) enlisted insect species from different families that enter into prolonged diapause mainly during pupal stage (Danks 1987, pp. 181-183). However, prepupal and larval stages are also common in the list. Longest diapause (5-10 year) has been found in the prepupa of *Prodoxus inversus* (Powell 1984). Surgeoner and Wallner (1978) found interpopulation variation in prolonged diapause in Lepidoptera.

1.1.7.1 Extended diapause in Thaumetopoeinae

Prolonged diapause is supposed to be typical of Thaumatopoeinae, a subfamily of Notodontidae (Halperin 1969). Superdiapause has been recorded in the species *Thaumetopoea pinivora* (Biliotti et al. 1964), *Th. processionea* (Biliotti 1953), *Th. wilkinsoni* (Wilkinson 1926), *Th. solitaria* (Halperin 1983) and *Th. pityocampa* (Besceli 1965, Biliotti 1953). Halperin (1969) reported failure of eradication campaign due to the prolonged diapause of *Th. wilkinsoni* in Israel. He found the rate of emergence to be highest in the first year and marked fluctuation in the following years. Up to 68% of the individuals of this species can enter prolonged diapause under laboratory condition. Pupal diapause may persist for six years in *Th. wilkinsoni* (Halperin 1969). Halperin (1983) found prolonged diapause of 1-2 year to be a common phenomenon in *Th. solitaria*. An interesting finding was variation of persistence of prolonged diapause in different condition. When he took the descending larvae in the pots, superpause either did not occur or lasted only for one year. But, transfer into the caged soil plot resulted in 2-year extended diapause. The observation was consistent with the study with *Th. wilkinsoni* (Halperin 1983). On the other hand, Halperin's (1990) experiment with *Th. wilkinsoni*, a sister species of *Th. pityocampa,* revealed 1-9 years of extended diapause and fluctuating emergence in the species.

1.1.7.2 Extended diapause in Thaumetopoea pityocampa

Pine processionary moth (*Th. pityocampa*) typically enters into prolonged diapause at high elevation and latitudes. Superpause may occur in this species after biotic and abiotic trouble in population growth (Démolin 1969). In addition, Tamburini et al. (2013) assumed the prolonged diapause to be an explanation of negative density feedback in the pine processionary population dynamics. However, biotic and abiotic factors behind the prolonged diapause of this species still remain unknown. On top, deciphering the prolonged diapause strategy of pine processionary moth is particularly critical since it has been described as a factor increasing invasion speed in stochastic environments (Mahdjoub & Menu 2008). Adding insult to the injury, Battisti et al. (2005) demonstrated the latitudinal and altitudinal expansion of pine processionary moth due to increased winter temperature.

Masutti and Battisti (1990) discussed the life cycles of pine processionary moth where they identified few parameters controlling the population cycles. Battisti (1989) assumed the higher percentage of pupal diapause during decreasing population level compared to increasing and outbreak population. In addition, fecundity and percentage of female (sex ratio) were presumed to be decreased while activity of natural enemies increased during the falling population period. Moreover, they opined longer adult emergence period (2-3 months) with 2 or 3 peaks to be the characteristic of declining population.

Masutti and Battisti (1990) also hypothesized the duration of feeding period in larvae to be correlated with the variations in the percentage of pupal prolonged diapause in different population cycles. To them, food or climatic conditions prolong the feeding period of the larvae, thus percentage of prolonged pupal diapause. Halperin (1983) reports temperature to be main factor for the length of larval development where, larvae hatched in February stops feeding after 50-60 days while larvae hatched in March get warmer weather and enough food thus complete development within 42 days. Nevertheless, percentage of individual entering prolonged diapause is negatively correlated with soil moisture, thus rainfall (Markalas 1989). Increased activity of fungal parasitoids with increased moisture and temperature could be a reason for this. But, we assume, this happens rather due to the mortality, not for prolonged diapause itself.

Démolin (1990) discussed the mechanism of diapause breaking of pine processionary moth (see ANNEX 2). He assumed that both diapausing and non-diapausing pupae resume their developmental activity about 40 days prior to emergence, a time that he called “key period”. In this period, both type of individuals start oogenesis, but pupae destined to prolonged diapause degenerate oocyte right after formation.

It was possible to manipulate the emergence time and even the intensity of prolonged diapause through putting the pupae in different temperature during and before this key period. Both high (≥ 30°C) and low (12-14°C) temperature can slow down the development and thus emergence time in pine processionary. It is also possible for one type of population to switch into another due to the variation in temperature.

Although we don't know how he did his experiment, we clearly have some intriguing ideas to proceed on. If his ideas are true, it will have significant importance in the understanding of ecology of pine processionary moth. Apprehending the mechanism of temperature in key period may allow us predicting population trend of pine processionary moth in the next year.

Apart from the temperature, rainfall may have a significant role in the seasonal ecology of pine processionary moth. Tauber et al. (1998) hypothesized moisture to be potential token stimulus, developmental modulator and behavioral cue for diapause related stages. In their earlier paper on Colorado potato beetles, Tauber et al. (1994) found the emergence of beetles to be highly associated with moisture at the soil surface. However, they conclude that, after adequate heat accumulations, moisture acts as a behavioral stimulus for emergence from the soil.

1.2 Objectives and research questions

Since the metabolic activity continues during the diapause, though at a reduced level, insects loose energy over time. Although generalization is not easy, it can be said that insects need to accumulate energy reserves before entering into prolonged diapause to ensure post-diapause survival, fecundity and flight capability for dispersal. It is therefore a logical perception that individuals with higher size and energy reserve can enter prolonged diapause. It can thus be presumed that individuals with smaller size and energy cannot enter prolonged diapause, or, if they enter, they would not be able to maintain as long as the bigger ones. Therefore, a threshold of size or energy might exist, which determines whether diapause is entered or not, as well as, if entered, how long should it be maintained. Insects can assess their internal level of nutritional reserve (see review by Hahn and Denlinger 2007). The maintenance of prolonged diapause could thus depend on the availability of resources, in relation to a given threshold level.

1.2.1.1 Timing of emergence in diapausing and non-diapausing individuals

Earlier emergence can be beneficial for insects, for example, ability of males in competing for females with other males. Earlier emergence can also help insects to find extra period of time for consuming food, thus accumulating extra reserve of energy. However, environmental cues and host status might regulate this phenomenon. In pine processionary moth, emergence occurs earlier at colder conditions (higher latitudes or elevation), and is often associated with occurrence of prolonged diapause. This is thought to be an adaptation to cope better with harsh winter conditions, i.e. having more developed larvae when winter starts. We assume that earlier emergence is associated with prolonged diapause, while non-diapausing individuals emerge later and are thus incurring in higher risk of winter mortality.

1.2.1.2 Size of diapausing and non-diapausing individuals

Pine processionary moth is a summer diapausing insect, and prolonged diapause is common in this species. As the temperature is a powerful regulating factor for the speed of metabolism, the issue of summer diapause in this temperate species is more critical. We presume the threshold of size, thus internal energy reserves, plays an important role in diapause entrance and intensity in this species. Prediction is that non-diapausing individuals are smaller than the diapausing ones, although this difference should disappear at emergence because the diapausing individuals are consuming the extra energy during the additional time spent in the soil.

1.2.1.3 Intensity of prolonged diapause and rate of emergence in cages

A cage experiment was setup in Venosta for monitoring the extended diapause in two cohorts of the pine processionary moth. The annual survey of the emergence has allowed identifying the number of individuals emerging each year, the timing, and the total duration of prolonged diapause.

1.2.1.4 Population dynamics in Venosta

Data of historical pheromone trap catch and pest suppression operations can be used to assess the trend of population of the pine processionary moth in Venosta. Regulation and ecology of prolonged diapause should be reflected in the analytical results of regular trap catches, for example by detecting different peaks in emergence timing (see 1.2.1.1). In addition, we can understand how important prolonged diapause is in the sustenance of population dynamics when pest suppression operations are conducted over large areas.

1.2.1.5 Environmental cues and diapause control

We discussed the responsibility of different environmental and internal cues to insect diapause in our review. Timing of first emergence every year should be related to those key players. As a preliminary experiment, we would like to test the function of two potentially most important abiotic factors in the timing of emergence. Therefore, our final objective would be to assess the role of temperature and rainfall in the first emergence date assumedly typical of diapausing individuals of processionary moth in Venosta.

2 Methodology

2.1 Study area

The Venosta/ Vinschgau Valley is situated amidst the Southern Alpine region of South Tyrol at the borders of Italy, Austria and Switzerland. It has an east–west orientation with north-south facing slopes (Battisti et al. 2005). Venosta Valley is characterized by its unique arid climate and more than 300 days of sun per year (http://www.venosta.net/en/welcome.html). It's a typical Inner-Alpine dry valley with less than 600 mm of annual precipitation (http://www.c3alps.eu/index.php/en/vinschgau-val-venosta-it-sidemenu).

Figure 1: Position of pheromone traps and their elevation. A rearing cage is also available near traps of Sportplatz. (Source: Google Earth)

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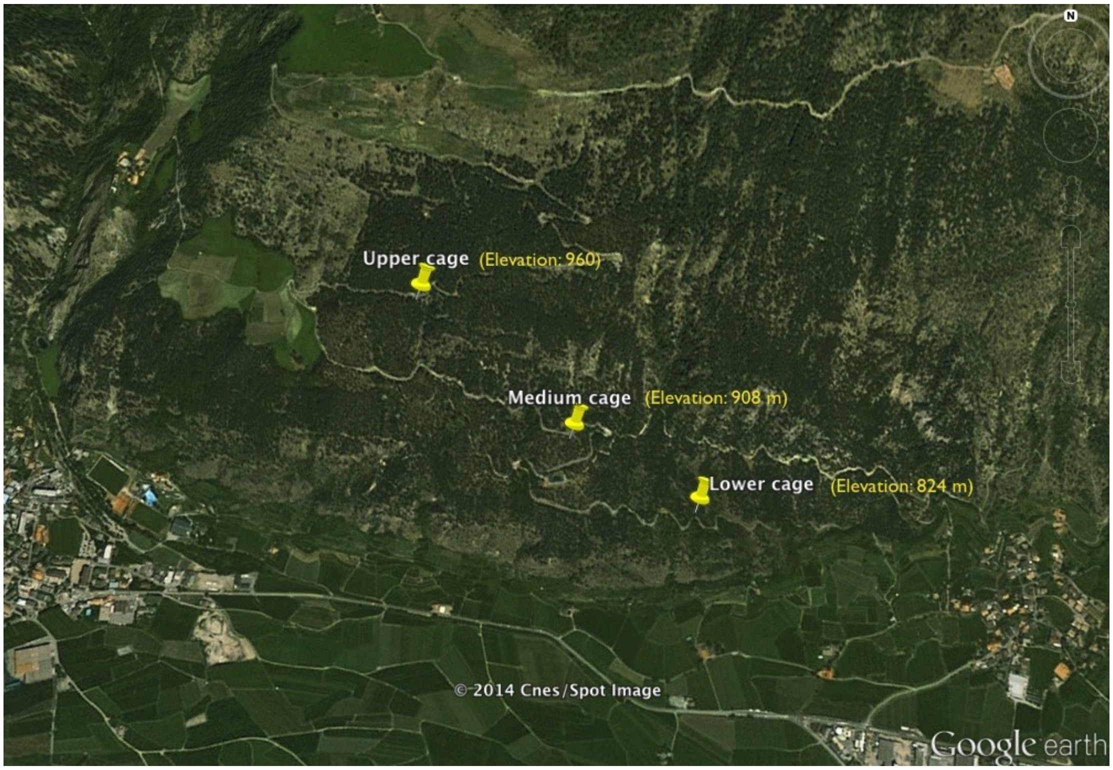

Figure 2: Position of the cages in Vezzaner and their elevation. (Source: Google Earth)

Venosta valley represents the upper edge of the range of pine processionary moth in the central Alps. Although historical occurrence of the insect is documented during the 20th century, it was only since 1998 that a large outbreak started and the population colonized the upper parts of the valley and the upper slopes, reaching an elevation as high as 1500 m on the south-facing and 1200 m on the north-facing slope (Battisti et al. 2005). Since then the population has fluctuated in density (Zovi et al. 2006), and plant protection measures were adopted (Aimi et al. 2006). Climate change has been invoked to explain insect survival at elevations that were considered prohibitive for the insect based on previous knowledge. Recent work, however, has documented that the thermal ecology of this species is more complex than expected and that local adaptation to climate can be observed (Hoch et al. 2009).

As soon as the outbreak occurred, the local foresters started the pest surveillance by mapping the infested areas, deploying a pheromone trap network, and installing large cages for the monitoring of the prolonged diapause intensity. The latter issue was started because it was expected that under extreme climatic conditions most of the pupae would enter prolonged diapause (Démolin 1969).

2.2 Methods

2.2.1 Catch data from the pheromone trap

Figure 3: Pheromone trap

Fifteen pheromone traps were deployed for capturing male moths in three different sites namely, Vezzaner Leiten (we will dubb this only Vezzaner later on), Priel, and Sportplatz, in the order of increasing elevation, with 5 traps in each site. Capturing started in 1999 and continued till present. In 1999 the experiment started in each site with three traps developed by the Forest Service of Trento district, consisting in a funnel-trap with large wings above it, and two traps marketed by Serbios as Super Green (http://www.serbios.it/), which is a classical funnel-trap for large moths.

The Forest Service traps were progressively replaced by the Serbios traps so that from 2007 only Serbios traps were used. Foresters visited traps every week during the emergence period and recorded the data. From 2013 foresters were asked to preserve in ethanol 95% all the male moths that were alive at the time of the check.

2.2.2 Prolonged diapause experiment

During winter 1999 three large cages (2x2x2 m) were constructed at Vezzaner Leiten over an elevational gradient (824, 908, 960 m) and one cage was set up at Sportplatz (near pheromone traps, with an elevation of 963m). The cages have a wooden frame and a wire net (mesh 2 mm) and the walls enter the soil down to a depth of 30 cm (Figure 4). In April 1999, a total of 300 nests were collected from the same site at an elevation of 800-1000 m. Nests were then randomly assigned to each of the three cages, trying to get the same number of larvae per cage. This was done based on the assumption that larger nests host about 200 larvae. Overall, a number of larvae estimated to be between 4000 and 6000 were added to each cage. The larvae were provided regularly with pine branches as food until pupation, which was inferred from the time when no more needles were eaten. The leftover was then removed and the cages were regularly inspected with a weekly frequency during the rest of spring and during summer. Emergences were then checked with the same frequency during each of the following years, and stopped when no emergences were observed for three years (2009). Foresters counted the number of individuals irrespective of the gender and did not preserve them. Soil samples were then taken from inside the cages and no cocoons were found.

During winter 2013 the soil inside the cages was checked again and no cocoons were found. We then decided to start another cohort of rearing by using nests

Figure 4 Cage in Venosta (elevation: 908 m)

from the same area as in 1999. As in 2013 the population density was very low, we collected nests to start two of the three cages, namely at the elevation of 800 and 1000 m. The number of nests, and that of the estimated larvae, was the same as in 1999. In 2013, however, we asked foresters to count separately male and female moths, and to preserve them in ethanol 95% for further analysis. The frequency of the inspections was the same as in the 1999 cohort.

2.2.3 Analyses carried out in relation to the objectives

2.2.3.1 Timing of emergence in diapausing and non-diapausing individuals

By comparing the emergence time in the cages with the catch in the pheromone traps we may infer if there are differences in timing between diapausing and non-diapausing individuals of the pine processionary moth. Both cages and pheromone traps were checked on the same day by the same person, reducing thus possible errors. In both cases, both alive and dead individuals were counted and their emergence was attributed to the days since the last check. We have emergence data of pheromone traps alongside current year emergence ones for the cohorts of 1999 and 2013. We construct the catch curve of those pheromone traps and found conspicuous bimodality or multimodality. Therefore, it is presumed, those that created earlier peaks are from diapausing individuals whereas individuals creating later peaks would be either from current or previous year. This is confirmed from the data of current year emergence in pheromone traps and cages. We found emergence in the pheromone traps before the first moth emerged in the cages. Therefore, we are sure that, those individuals in pheromone traps, which emerged before the first emergence of cage, are from diapausing cohorts.

2.2.3.2 Size of diapausing and non-diapausing individuals

As we know that moths emerged from the cages in 2013 are surely non-diapausing, because the experiment with a new cohort has been set up this year, we obtained an estimate of body size of moths by measuring the length of the foreleg femur. On the other hand, we expected a mixture of diapausing and non-diapausing moths from the pheromone traps. After we took them in our laboratory, we measured the femur length of forelegs from male moths. We could thus compare the lengths of males from the cages with those from pheromone traps. Femur length was considered to be a proxy of body size (Shingleton and Foster 2001). For measurement, we first took each single moth onto a Petri dish beneath the microscope with graduated lens. After that, we split one of the forelegs from whole individual keeping the magnification of microscope at 10x. For measuring the femur length, we used the magnification of 25x. The measurement starts from the end of tibia until the very start of trochanter (Figure 5). Thereafter, we put the record in an excel file for further statistical analysis.

2.2.3.3 Environmental cues and diapause control

We collected weather data from local meteorological station of Silandro/Schlanders. In addition, for the larval population density, we used nest census made by the Forest Service. Thereafter, we analyzed the data statistically using statistical tools

Figure 5: Measurement of the foreleg femur length

2.2.4 Surveillance of spraying operations

We obtained data about spraying operations with a commercial preparation of *Bacillus thuringiensis kurstaki* (Foray 48B) from the Forest Service. In the surveyed area, spraying was carried out in autumn for the cohorts from 2000 until 2007. In the years when the insect was particularly abundant and the autumn spraying was not enough to control the pest, the spraying was repeated in spring. In general, the spraying had efficiency higher than 90%.

2.2.5 Literature survey

During the literature survey I mainly relied on books and published articles in the scientific journals. For searching related papers, I mainly used SCOPUS, Web of Knowledge and Google Scholar search engines. I selected few key words (see below) and searched them using search engines. Search engines, mainly SCOPUS and Web of Knowledge, led me to various articles. I first looked at the titles and abstracts. Whatever, I found relevant to my interest I downloaded and read them. Most of the articles are common between search engines, but few articles are uncommon among them.

In addition, I followed "backward spider" approach of searching literatures. It means searching literatures from the reference list of important articles. This was very effective in my literature search. Moreover, "sideway spider", means the related article links in the search results of search engines mainly in SCOPUS and Web of Knowledge, helped in finding interesting literatures. For some rare articles in language other than English, I used personal collection of my supervisor, Professor Mr. Andrea Battisti of Department of Agronomy, Food, Natural Resources, Animals and Environment of University of Padova.

Few keywords I used are: "why study diapause", "list of diapausing traits", "diapause biomarkers", "extended diapause in insects", "ecology of extended diapause", "ecology of prolonged diapause in insects", "theory of prolonged diapause in insects", "pheromone trap and diapause" etc. Moreover, I searched with "Danks" in the author section of SCOPUS that led me to works of renowned entomologist H.V. Danks. On the other hand, books on diapause were available at hand in the library of my department.

2.2.6 Standardizing weekly data in diurnal ones

With the trap data, we have standardized the weekly data into diurnal one through dividing the number of catch by the days it has been taken for check. We have considered the 15th of June as the standard date of emergence. To be able to count the diurnal catch of the first record in each year, we have subtracted 15 from the day of first check. For example, if the first emergences check is in 25th of June, we have subtracted 15 from 25 and then used the 10 to divide the number of catch. As an exception, we divided the number of catches by 7 for the first week of year 2007.

2.2.7 Standardizing trap type data

There are two types of traps in our dataset what we call "Old" and "New" type. The number of catches clearly varies between old and new types. Therefore, we needed to standardize both of them into "New" one in order to be able to compare the yearly data. First, we got the ratio of average diurnal catches between Old and New traps of each year for each site. In a new column, we put the values of the ratio for each site and year. Finally, we divided the average mean diurnal catch of all Old traps by their corresponding Old to New ratio. We recorded the result into a separate column as standardized mean diurnal catch. Now, record of old traps are standardized with that of new traps.

2.2.8 Correction of Pivot table data

We have few missing values in the dataset (Priel 2005 & 2006; Sportplatz 2007; Vezzaner 2010). While making the graph of population density over the years, the values of total year with those missing values disappear. Therefore, we have corrected it manually by taking the average of "standardized mean diurnal catch" of those traps with their corresponding years.

Table 4: Example of how pivot table was corrected

Site	Year	No. of Observation	Sum of Observation	Corrected mean catch
Priel	2005	54	109.45	2.026851852
Priel	2006	50	69.70	1.394
Sportplatz	2007	60	71.80	1.196666667
Vezzaner	2010	39	99.08	2.540512821

2.2.9 Statistics and software

For relating Julian date of first emergence with both temperature and rainfall data, we used simple linear regression. In addition, two-tailed, type-2 Student's t-test has been used for the comparison between different combinations of groups. All the statistical and graphic analysis has been done using MS Excel 2011 for mac (Microsoft 2010).

3 Result

3.1 Timing of emergence in diapausing and non-diapausing individuals

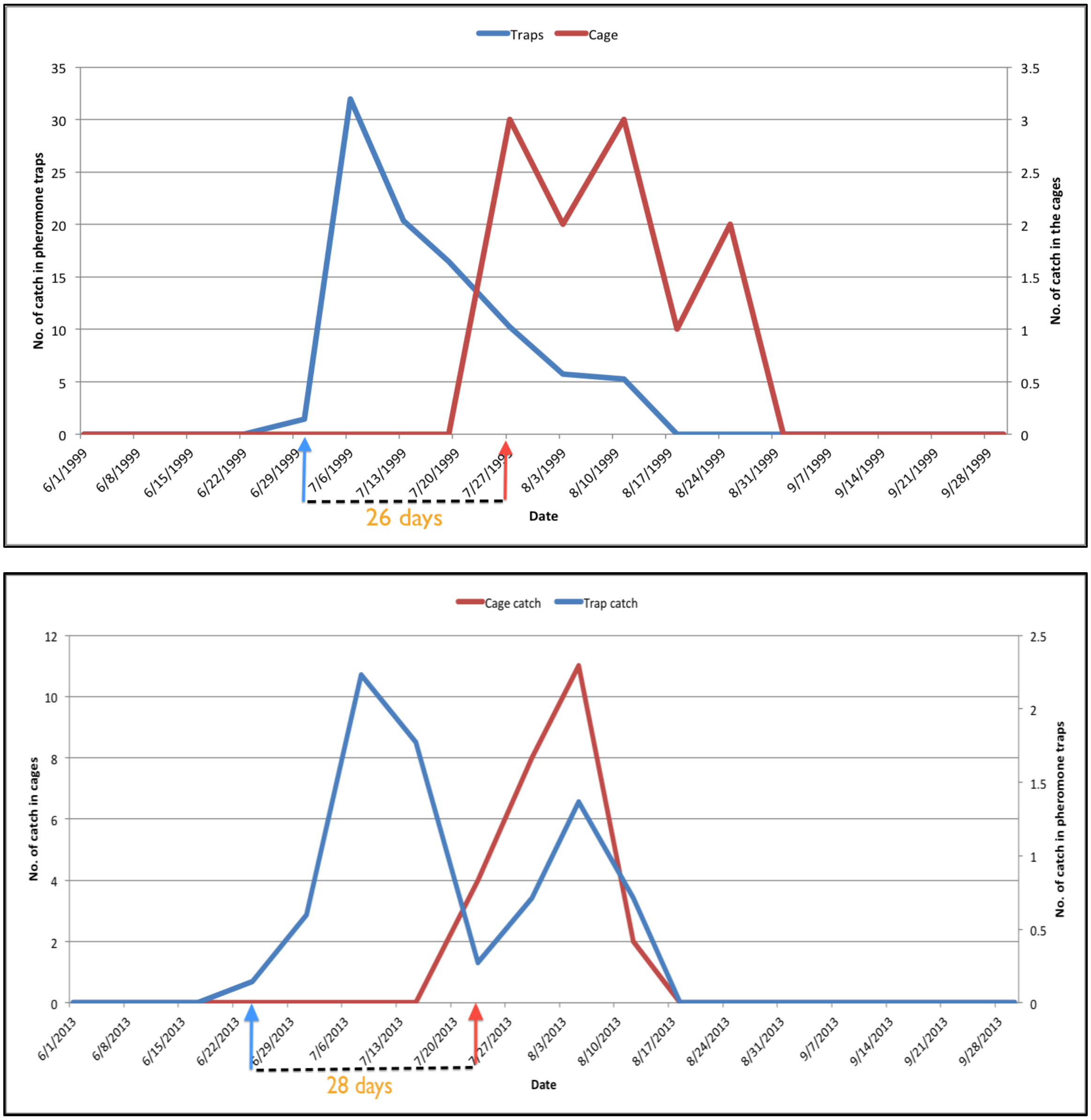

Figure 6: Number of males caught daily in pheromone traps and in the rearing cages for the 1999 (above) and 2013 (below) cohorts of the pine processionary moth in Venosta.

It is very tough to distinguish in nature whether a moth belongs to current year cohort or previous year cohorts. Experimental setup thus is necessary for proper identification. As it has been discussed in the method chapter, we have data of moth emergence from both pheromone traps and experimental cages for two cohorts, i.e. 1999 and 2013. In the pheromone traps, the problem of differentiating between diapausing and non-diapausing moths still prevails, since we almost never know which cohort they come from. The only certain way of identifying current year moth and their time of emergence is tracking emergence from cage setup. Emergence both in 1999 and 2013 corresponds to the current year emergence for both cohorts. Our basic idea is to check first emergence date from the cages of 1999 and 2013 and compare it with the initial emergence date in the pheromone traps.

In 1999, first moth in the cage was found on 27th of July. Meanwhile, first catch in the pheromone traps was recorded in 30th of June (Figure 6; above). A similar result has been found in 2013 as well (Figure 6; below). First moth flew in the cage on 23rd of July, while pheromone traps already lured inaugural moth on 24th of June that year. From the results, we are now sure that those moths that emerged earlier comes from previous diapausing cohorts. In addition, current year moths fly on average 27 days after the emergence of diapausing individuals.

3.2 Comparing the size of diapausing and non-diapausing individuals

We had three groups of moths for the analysis. Moths from the first group emerged naturally in the cages, corresponding to the non-diapausing current year cohort. Males of the second group were obtained from pupae collected from the same cage and taken into the lab, allowing them to be emerged in the laboratory condition. Finally, pheromone traps gave rise to our third batch moths, which held a combination of diapausing and non-diapausing individuals.

Our idea was to compare the size of moths among three groups. Since, pheromone traps capture solely the males, we discarded females from our analysis in other two groups.

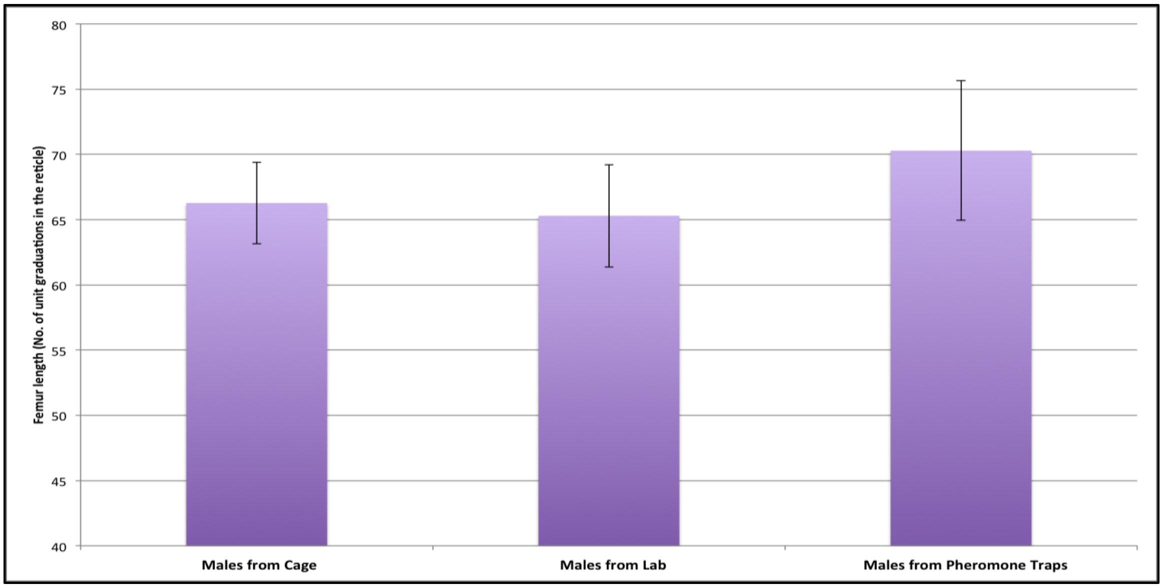

Figure 7: Major results of foreleg femur lengths of males from different groups. Males from cage and lab belong to the same 2013 cohort reared in the cage while males from traps are a mix of 2013 and previous cohorts [Bars represent mean femur length of male moths from different condition (error bars show standard deviation of the mean)]

We used two-tailed, type-2 Student's t-test for the comparison between different combinations of groups (e.g., cage males, lab males, earlier pheromone trap males (emerged before 23rd of July, 2013), later pheromone trap males (emerged during and after 23rd of July, 2013), pheromone trap together, and cage and lab together).

Table 5: Descriptive statistics of foreleg femur lengths of males

Statistical Parameter	Cage males	Lab males	Earlier trap males	Later trap males	Trap males together	Cage and lab together
Count	22	44	14	21	35	66
Average	66.27	65.30	69.07	71.10	70.29	65.62
Standard deviation	3.12	3.92	3.81	6.13	5.36	3.68
Standard error	0.67	0.59	1.02	1.34	0.91	0.45

Table 6: Student's t-test result for foreleg femur lengths of males from different groups

Comparison pair	*p-value*
Cage males Vs. Lab males	0.31
Cage males Vs. Trap males together	0.002
Cage males Vs. Earlier trap males	0.022
Cage males Vs. Later trap males	0.002
Lab males Vs. Trap males together	8.254E-06
Lab males Vs. Earlier trap males	0.003
Lab males Vs. Later trap males	1.966E-05
Cage and lab together Vs. Trap males together	1.306E-06
Cage and lab together Vs. Earlier trap males	0.002
Cage and lab together Vs. Later trap males	3.201E-06
Earlier trap males Vs. Later trap males	0.280

There was no significant difference (p=0.31) in the femur lengths between Cage males (n=22) and Lab males (n=44). Since both groups belong to the same cohort from the same cage, it was expected to show the similar results. We found no difference in the femur length from the earlier (n=14; from putatively diapausing cohort) and later (n=21; combination of diapausing and non-diapausing) pheromone trap males. It is because diapausing males get blended with the non-diapausing current cohort males in the later trap catches. Finally, analysis between mixture of cage and lab males (n=66) and mixture of pheromone trap males (n=35) resulted very significant difference (p=1.3059E-06). Notably, current year males from the cage and lab show on average smaller size (65.62±3.68) foreleg femur length than those in the traps (70.29±5.36) (Fig. 7).

3.3 Intensity of prolonged diapause and rates of emergence of adults in the cages

Duration of prolonged diapause in pine processionary moth has never been officially documented before under natural conditions. Set up of cages at Venosta was the first attempt of documenting duration of prolonged diapause and emergence pattern in pine processionary moth.

The upper cage of Vezzaner never produced any adults, the reason of which is unknown. Interestingly, all three cages showed different pattern in intensity of prolonged diapause and cohort dynamics. There was no first year emergence in Sportplatz cage. Diapause extended for seven years in the medium cage while lower cage showed the lowest extension (four years) among the three cages. Six year prolonged diapause has been observed in the cage of Sportplatz.

Distribution of emergence over years in the lower cage was positively skewed, while the medium cage showed more or less normal distribution. Although, lower and medium cages exhibited unimodal distribution of emergence over years, cage in Sportplatz displayed bimodal distribution.

Table 7: Total number of moths, either male or female, emerged in each year for the 1999 cohort in the cages

Year	Lower cage	Medium cage	Sportplatz cage
1999	9	2	0
2000	402	20	13
2001	100	25	87
2002	11	241	20
2003	7	406	70
2004	0	100	7
2005	0	38	1
2006	0	9	0
2007	0	0	0
2008	0	0	0

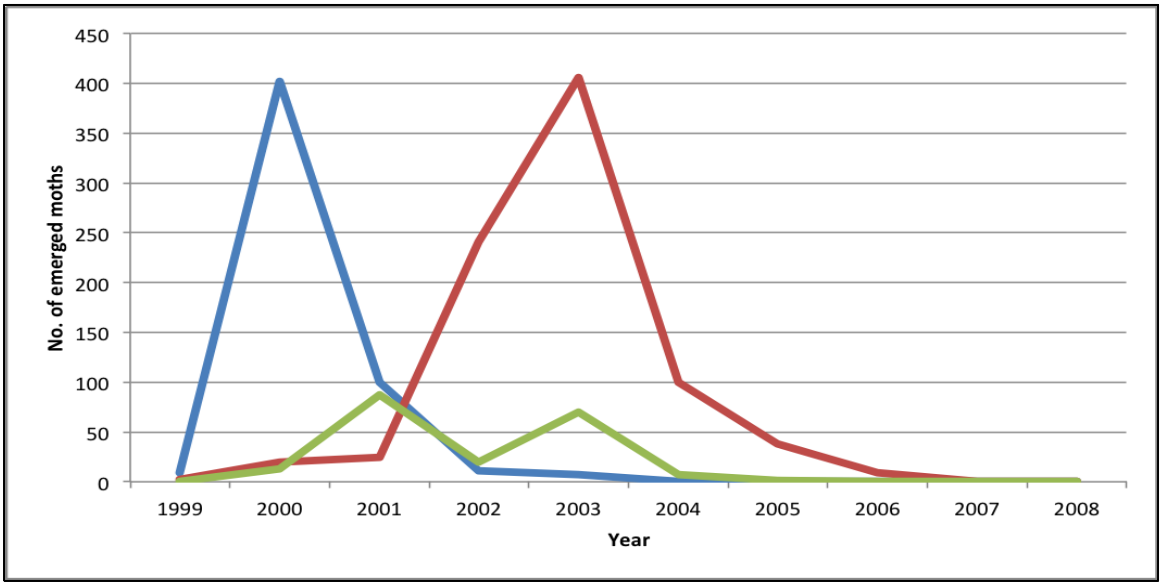

Figure 8: Distribution of moth emergence in the cages over years. Lower cage (blue coloured line), medium cage (red coloured line), and Sportplatz cage (green coloured line) are situated in the elevation of 824m, 908m, and 963m respectively.

3.4 Population Character

3.4.1 Population dynamics of pine processionary moth

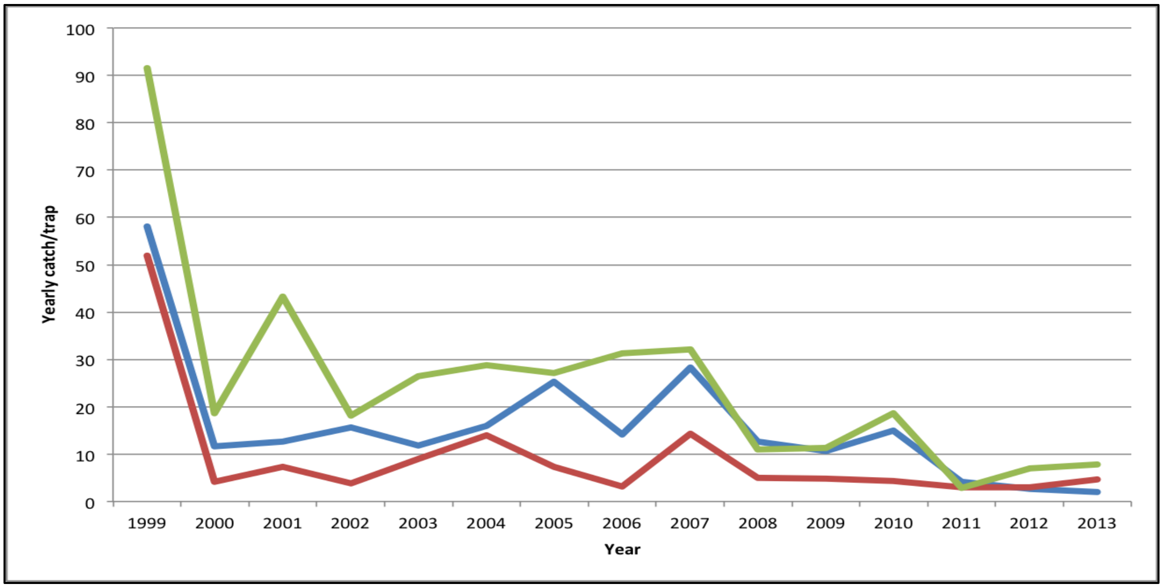

Figure 9: Mean number of males of pine processionary moth caught in pheromone traps at three sites (green representing Vezzaner, blue representing Priel, and red representing Sportplatz) in Venosta. Five traps were used at each site.

An irregular pattern is evident in the population dynamics of pine processionary moth derived from the pheromone traps' catches in Venosta. Density was very high in 1999, which dramatically dropped in the following years due to the application of Btk insecticide (see Discussion). Number of catches in pheromone traps varied according to altitudinal gradient. Pheromone trap catch duration ranged from 43 to 91 days. Therefore, density was consistently highest in Vezzaner, whereas, density remained mediocre in Priel and lowest in Sportplatz. Three phases of density is obvious from the graph. Year 1999 has the highest density, a medium density prevailed from 2000 to 2007, and the lowest density existed thereafter.

3.4.2 Yearly population abundance peaks

All three sites showed similar pattern in the catch distribution, although in different scales, only for the years 1999, 2006, 2008, and 2012. In other cases, distribution patterns differed among sites. Seasonal trends in abundance produce different types of peaks that can be attributed to few characteristics for different years (Table 8). Graphs of population abundance of all years have been attached in the ANNEX 1.

Table 8: Peak characteristics of yearly population abundance curves in three sites (V, P, and S stand for Vezzaner, Priel, and Sportplatz respectively).

Peak characteristics	Year (identifying sites)
One earlier major upright peak	1999 (V, P, S); 2000 (V, P, S); 2003 (V, P); 2009 (V, P, S); 2012 (V, P, S)
One earlier major flat peak	2006 (V, P)
One earlier major peak + one later minor peak	2005 (V, S); 2013 (V, P, S)
One earlier major peak + several later minor peaks	2001 (V); 2002 (V); 2004 (V); 2007 (V, P, S)
Almost similar earlier and later peaks	2008 (V, P, S); 2011 (V, P)
One earlier minor peak + one later major peak	2010 (P)

3.5 Overall analysis including weather

3.5.1 Diapause from cages and pheromone traps

We found seven years of prolongation in diapause of pine processionary moth in the cages. As Btk spray reduced the density of population dramatically following 1999, we supposed emergence thereafter at least until 2006 to be the contribution mainly of individuals from prolonged diapause of 1999 and earlier cohorts. We wanted to see if there is any positive correlation between the emergence pattern of cages and the catch of pheromone traps from 2000 to 2006. However, we did not find any strong correlation, rather it produced a negative correlation (r=-.58).

3.5.2 Extended diapause through time (%)

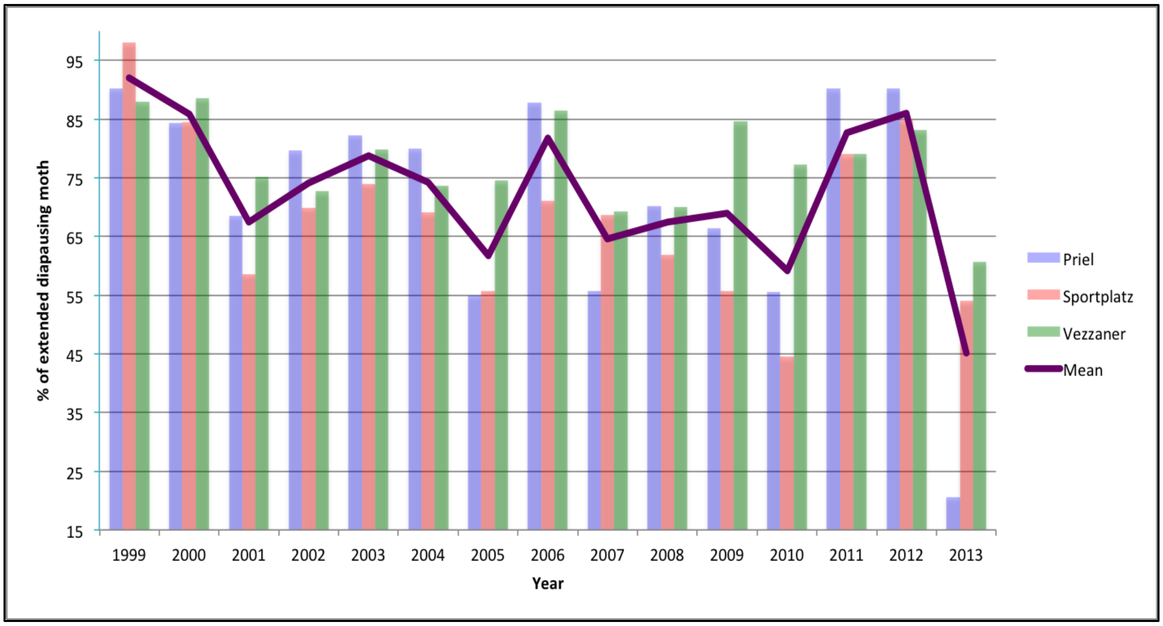

Figure 10: Percentage of male moths caught in the pheromone traps in the first 27 days of the catch period in each year (see text for explanation)

Considering the emergence timing result of our first objective, we hold first 27 days as the emergence period of only diapausing individuals in each year. Although, diapausing individuals can come out later as well, but we don't consider them since they mix up with the current year cohorts. We have calculated the percentage contribution of both sets of individuals coming from first 27 days and later. The result shows higher ratio of diapausing individuals those coming from first 27 days every year. However, contribution varies throughout years that can be somewhat attributed to an irregular pattern (see Figure 10). The outbreak year of 1999 showed highest percentage of contribution from extended diapausing individuals while 2013 revealed the lowest contribution of superpausing moths. In addition, 2002, 2003, 2006, 2011 and 2012 markedly resulted very high ratio of prolonged diapausing individuals. In contrast, in addition to 2013, lower contribution is evident in the year 2005, 2007, and 2010.

3.5.3 Thermal sum and first emergence

We wanted to see if first catch date in pheromone traps in each year can be explained by the corresponding thermal sum of different time scales. Simple liner regression between the first catch date (Julian date) and thermal sums (°C) resulted different strength of relations. Thermal sum since January to emergence date is the one explaining most of variability (53 percent). The 40% of variability in the first emergence date can be explained by thermal sum of 40 days prior to emergence date. Consideration of the thermal sum of previous 45 and 50 days, however, generated almost similar coefficients of determination (0.33 and 0.31 respectively) that are less than the earlier twos.

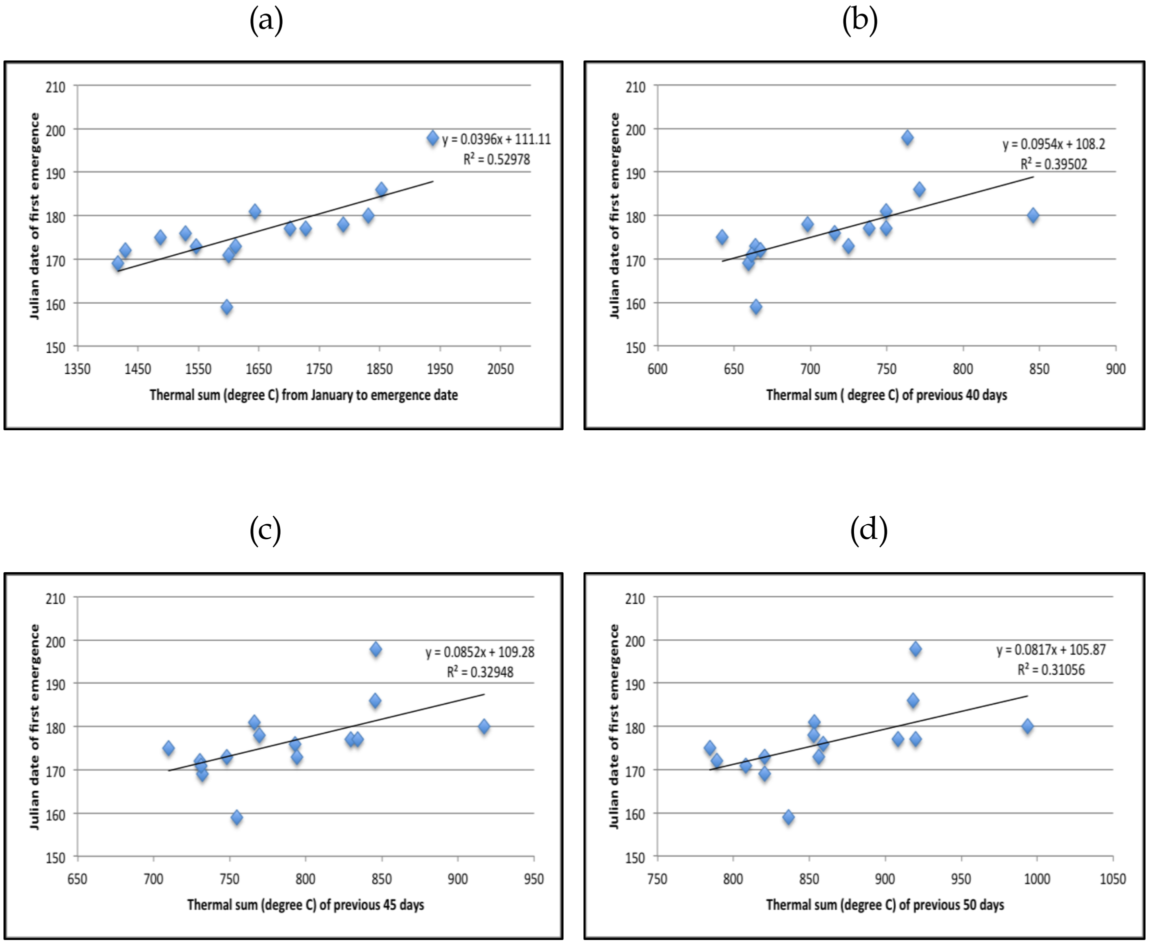

Figure 11: Relationship between Julian date of first emergence and temperature (°C) of (a) January to emergence date, (b) previous 40 days, (c) previous 45 days, and (d) previous 50 days.

3.5.4 Rainfall and first emergence

When we took rainfall of different scale, it turned out to be a good predictor variable for predicting first emergence dates of studied years. Rainfall since January to emergence date and of previous 50 days could explain the variation equally well (36%). Almost the same explanation of variation arose for rainfall of last 45 days (35%), while rainfall of 40 days preceding emergence, however, predicted the least amount (only 19%) of variability.

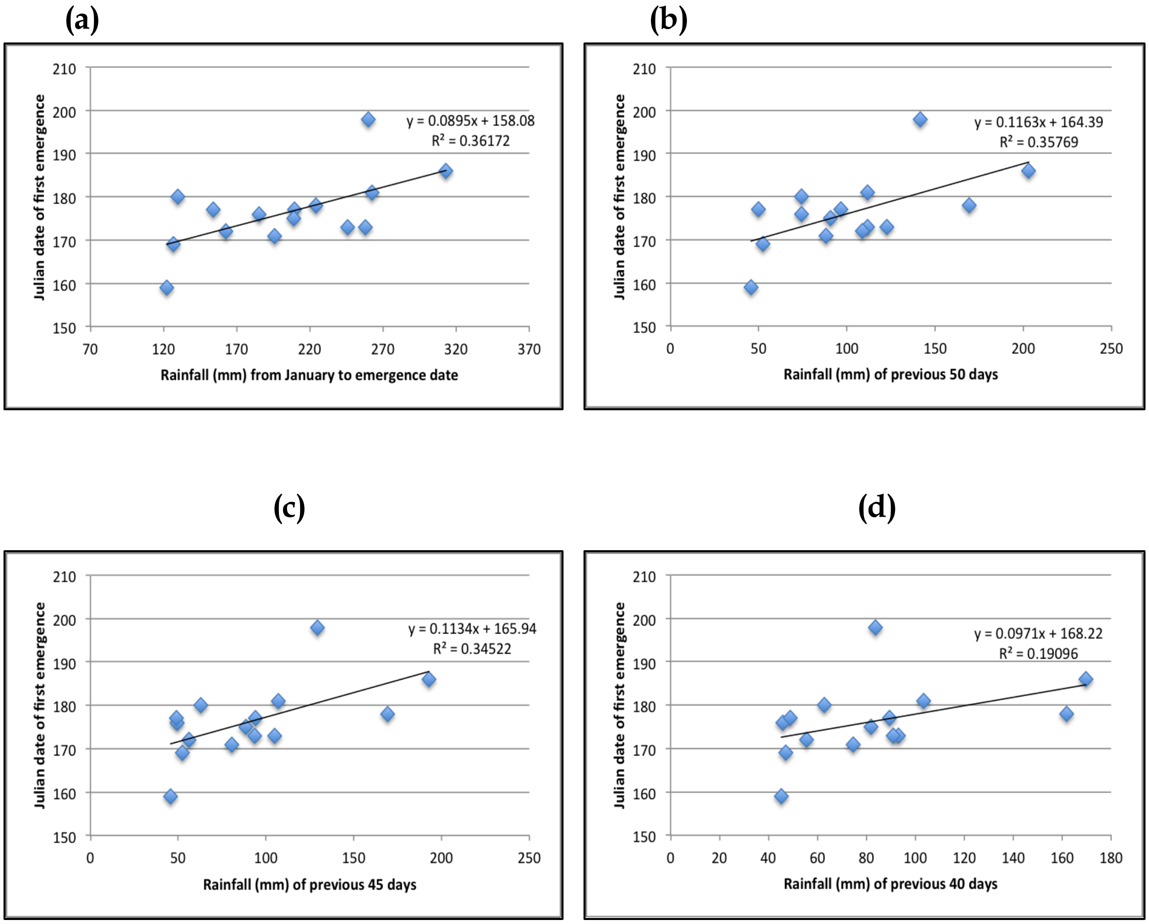

Figure 12: Relationship between Julian date of first emergence and rainfall (mm) of (a) January to emergence date, (b) previous 50 days, (c) previous 45 days, and (d) previous 40 days.

4 Discussion

4.1 Synthesis of results

That pine processionary moth in Venosta extends its diapause over several years is evident from our experiment result in the cages. Démolin (1969) reported the diapause of pine processionary moth to be extending up to six years under controlled, semi-field conditions. In Venosta, we confirm the intensity of extended diapause to be up to seven years. We found variation in the intensity of prolonged diapause among sites. This may happen due to the variation in temperature, where sites with higher temperature are prone to end the prolongation of diapause earlier than those with lower temperature. Halperin (1990) found the similar effect of elevation, a proxy of temperature, in the intensity of extended diapause in *Thaumetopoea wilkinsoni* Tams, a sister species of *Thaumetopoea pityocampa*. In his experiment, those that were reared in comparatively higher elevation (850 m) extended diapause up to nine years, whereas individuals reared in lower elevation (50 m) resulted only six years of extension in diapause. However, our Sportplatz cage (with highest elevation of 963 m) showed lower intensity in prolonged diapause than that of medium cage (908 m). This anomaly could arise either due to inadequate size of experimental cage or for higher mortality. Halperin (1990) documented higher mortality in the upper elevation site (46.8%) than the lower elevation site (22.3%). In addition, if the rainfall related hypothesis discussed later is true, it might have a role in this anomaly. Variable outcome of intensity in different cages within very short distance and temperature variation, along with putative energetic constraint stemming from limited food source inside cages, in our site lead us to expect even more intense prolonged diapause on site.

Emergence pattern of extended diapausing insects can be decreasing or fluctuating. We find a fluctuating pattern in the experimental cages of our study site. Similar fluctuating pattern was evident in the *Thaumetopoea wilkinsoni* (Halperin 1968). This fluctuation may have arisen in response to certain environmental cues.

Spread of emergence duration ranged from 43 to 91 days. In addition to the role of environmental factors, this variation might arise from difference in individuals' ability of physiological and behavioral response (discussed in later section).

After the outbreak took place in 1998, local forest department started aerial spraying of *Bacillus thuringiensis kurstaki* (Btk) over infested area of Venosta. Until 2007, core areas were sprayed regularly with the insecticide, which was later limited until 2010 only to the areas prone to human hazard, e.g., adjacent to the roads and paths, and was stopped completely thereafter (Figure 13). Although, the sprayed area varied over years, Vezzaner (where the lower and medium cages are located) got sprayed regularly. Efficacy of Btk spray was very high (Zovi et al. 2006 and unpublished data from Forest Service). Logically, we assume the contribution of current year cohort in the population density of pine processionary remained very low after 1999. Those that emerged until at least 2006 should be the contribution mostly of diapausing individuals of 1999 and earlier cohorts. There could be input from the diapausing cohorts (at least 7 years) before 1999 as well, but as we know, outbreak took place in 1998 and reached peak in 1999, contribution of those previous cohorts should be minimal. If we consider both the cut-off of Btk spray and intensity of extended diapause in the density of pine processionary population, current year cohort should reappear after 2007. However, our result shows dramatic drop of population from 2008. This might happen due to the establishment of natural enemies (egg parasitoids) on site (Zovi et al. 2006). Zovi et al. (2006) documented unexpected increase in the density of egg parasitoids of pine processionary (mainly *Baryscapus servadeii*) from 2003, which resulted around 50% increase in mortality of eggs of the population in the following years. Notably, parasitoids took years after outbreak (1999) to establish successfully on site in spite of high host density. Zovi et al. (2006) conjectured this phenomenon as a consequence of problem in synchronization with host at the beginning, which was overcome owing to the process commenced by the record warm summer of 2003 (Luterbacher et al. 2004). In conclusion, Zovi et al. (2006) expected a further increase of mortality arising from the reduced initial colony size of pine processionary stemming from the mortality by natural enemies. Despite the combination of successful establishment of parasitoids from 2003 and spray of Btk since 1999, we observe continuously equal density of the population until 2007. This higher density can only be explained by the extended diapause of pine processionary spreading out from the cohort of 1999.

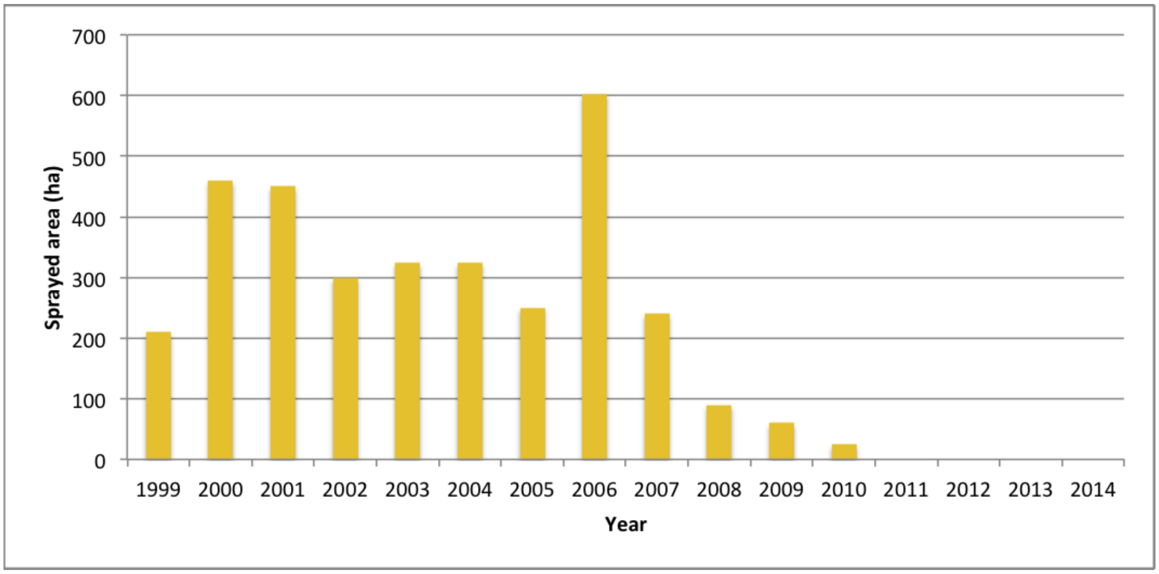

Figure 13: Amount of sprayed area with Btk in Venosta (unpublished data from Forest Service)

Interesting to note, considering the population dynamics and yearly-sprayed area, we can infer a threshold of population size below which spray can be avoided. In our site, amount of spray started getting reduced after 2007 according to the waning level of population, even though the spray was not stopped. When the yearly catch per pheromone trap went down to 10 individuals, forest department stopped spraying. This threshold should be attributed to health impact rather than economic one.

Trend of population density of pine processionary moth always remained unpredictable (Battisti 1989, Masutti and Battisti 1990, Aimi et al. 2006, Tamburini et al. 2013). Even pheromone trap catch did not appear to be a strong predictor of nest density in the next year (Aimi et al. 2006), although a general positive pattern has been found by using a specific trapping design (Jactel et al. 2006). All previous bewildered authors held extended diapause as a confounding factor, in addition to possible other factors, in the gap of their knowledge. Our attempt to correlate the emergence pattern in the cages with corresponding pheromone trap catch remained unsuccessful. The reason behind might be the space limit in the experimental cages. In addition, we saw that, all three cages reproduced three different distribution patterns. On the other hand, attraction range of a single pheromone trap can extend up to hundreds of meters (Schlyter 1992, Zhang et al. 1996). Consequently, inappropriate scale produced correlational anomaly.

Nevertheless, our study generated some primary information on the extended diapause of pine processionary moth in Venosta. We have been able to identify the gap in emergence between superdiapausing and current year cohort. Extended diapausing individuals always had an earlier emergence of average 27 days, which is consistent with the result of (Menu 1993). This result allowed us to decipher (though conservatively) the ratio of contribution from the extended diapausing individuals. The ratio of contribution from diapausing individuals throughout the study period was striking. Although the ratio of extended diapause was overestimated due to the suppression of current year population, the produced result implies how this species may overcome the possibility of extinction from lethal temperature and starvation in every cohort through spreading the risk of reproduction over several years.

Management strategy of pine processionary moth in Venosta after the outbreak was keeping the population density low by the application of Btk in the selected hotspot tourist areas along the roads and at the same time relying on natural enemies. So far, the strategy has earned significant success evident from population dynamics data. In addition to the highly effective spray of insecticide, natural enemies have established on site. However, expansion risk of outbreak area of this species in this region is very likely due to climate change through the process explained by Battisti et al. (2006). It is evident from the study of recent past, very quick response to climate change allow pine processionary go into outbreak density beyond its previous range. Undoubtedly, extended diapause bolsters its success helping pine processionary to avoid unexpected risk from biotic and abiotic enemies.

Tackling of pine processionary moth in cost-effective way might not be possible in the future. The promising way to face the upcoming problem lies greatly on how much we could decipher the mystery of extended diapause in this species.

4.2 Demolin's legacy

Post diapause quiescence is not expected in the aestivation diapause due to the presence of suitable environmental conditions for development in this case. Several symptoms of diapause termination are thus offered to explain such pattern. Increase of metabolic rate about two months before formation of oocytes is one of the criteria explaining the termination of diapause (Hodek 1996). However, designation of timing of diapause termination depends on which criterion is adopted (Tanaka et al. 1987).

Démolin's idea of "key period" actually corresponds to the idea of aforementioned symptom of diapause termination during the designated termination period. He considered start of oogenesis 40 days before emergence as the morphological marker of diapause termination. Forty days here can be attributed to the termination of diapause period and start of the post-diapause development (see Introduction). Although thorough test is needed to conclude about his ideas, we made a preliminary effort in explaining this process of extended diapause in pine processionary moth. Variation in first emergence dates among each year should be explained by possible responsible factors of extended diapause. As supposed by the Démolin's idea of "key period", pine processionary moths should resume or pause further development after the obligatory diapause in response to the temperature sensed during or before that period. Therefore, temperature of those periods should play the main role. Although he marked 40 days before emergence as the key period at 20°C, duration can vary according to variation in temperature. Therefore, considering the temperature of our study site, we ended up taking both thermal sum and rainfall of 45 and 50 days in addition to 40 days prior to emergence as the predictor variables. We also included estimation from January to emergence date each year to see the long-term effect of variables. The reason behind restricting the measurement until emergence date is simply because additional measurement would be confounding as it does not have any role after first moths emerge.

The mechanism of diapause maintenance and breaking is largely unknown. Démolin summarized his life-long experiments in a synopsis published in 1990. He treated the pupae with different temperature regimes, and concluded the following suppositions.

I. If you put the pupae at very low temperature (4 to 6°C) before key period, they stop development. This stop at development could be analogous to quiescence (defined in the introduction chapter).

II. If you put the pupae at temperature ranging 12 to 14°C before key period, prolonged diapause will be induced, that is, they will postpone their emergence to the coming suitable years.
III. If you put them into 20°C after the diapause is broken (the so-called 'key period'), they would emerge after 40 days. In other words, normal annual development will be obtained.

Our data about temperature, seen as a thermal sum, and development into adult, from pheromone traps, do not add any information at this regard, but they confirm the general rule developed by Démolin in his pioneer work of 1969. According to his model (Figure 14), the emergence time of adults is advanced, i.e. earlier in the year, as long as latitude or elevation increase. This is going against all predictions about development rate and temperature based on models developed for insect of temperate climate, and especially for those growing at low temperature (Huey 2010).

According to the general thermal performance curve, performance increases gradually with temperature up to some optimal level but then declines dramatically as temperature approaches lethal maximum limits (Huey and Stevenson 1979). Huey (2010), in his discussion about evolutionary physiology of insect thermal adaptation to cold environments, concluded that primary adaptation of thermal fitness curve to cold and fluctuating environment is associated with tolerance of extremely cold temperature, but quite slightly with shift in optimal temperature, performance breadth and maximum critical temperature. In ectotherms, evolution of optimal temperatures is very conservative (Hartz et al. 1983, Hyey et al. 2003). This happens either due to fitness reduction or minimal change in temperature with latitude during insect activity (in summer). In addition, range of temperature over which insects shows higher performance has no dependency on latitude, which might be a general pattern in terrestrial ectotherms (van Berkum 1986). Tolerance range, however, increase with latitude.

Unfortunately Démolin does not support his view with experimental data. Our data indicate that the same is true within the same locality by using years with different temperature conditions. As expected by the Démolin model, adults emerge later in warmer years. The variation explained by temperature alone varies between 31 and 53%, depending on the length of the period use for the calculation of thermal sum. In particular, the explained variation increases when the whole period from January to emergence is considered, indicating that accumulation of heat starts earlier in the year, and in this case the breaking of the prolonged diapause should occur also earlier.

The mechanism by which the growth is faster at lower temperature can be explained by a dome-shaped relationship between development rate and temperature, where maximum rate is obtained at relatively low temperature. In the case of the pine processionary moth, such a relationship is functional to the colonization of cold environments, with the anticipation of the feeding period to the summer and early autumn. Conversely in warmer habitats the high spring and summer temperatures slow down the development of the pupae and the emergence occurs in late summer and autumn, because winter conditions are not limiting for feeding.

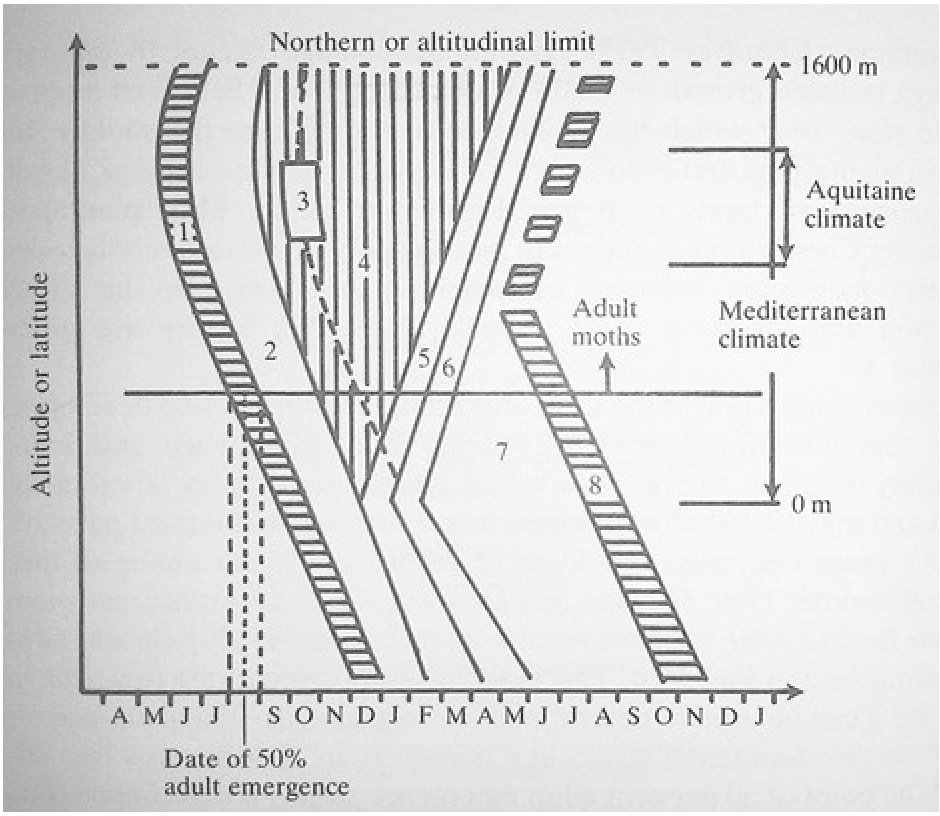

Figure 14: The life cycle of *Thaumetopoea pityocampa* in France in relation to the change in altitude and latitude. Letters on horizontal axis indicated initial letters of months in English. 1 = adults; 2 = pre-hibernation eggs and young larvae; 3 = pre-hibernation processionary larvae moving to overwintering sites; 4 = hibernation (it's not quite true since minimal activity is found in this phase despite low temperature) in nests; 5 = post-hibernation development of larvae; 6 = post-hibernation larvae heading to pupation sites; 7 = pupal diapause; and 8 = Adult development inside pupae. (Reference: Démolin 1969 from Speight and Wainhouse 1989)

Other factors should in any case explain the residual variability. Among these, rainfall seems to be an interesting one and our data go in this direction as rainfall is positively correlated with the Julian date of first emergence, i.e. adults emerge later in rainy years, irrespective of the period of time considered.

Water is often considered as an essential factor for diapause completion (Hodek 1996). According to him, water or moisture in aestivation diapause can be represented to the temperature threshold needed to break the post-diapause quiescence in the hibernation diapause. Let us clarify a little bit. In the colder regions, diapausing insects don't emerge right after breaking of diapause due to unfavourable lower temperature. Following the termination of diapause, they are believed to stay in a quiescence phase until the environment gets warmer, that is favourable for coming out. Although, summer diapausing insects don't have such sort of limitation of colder temperature, rainfall might act as threshold signal for them to start overt development (for more clarification, see first scheme of diapause development pathway described by Hodek 2002).

Concluding remark about rainfall could have been manifold. First, dryness or lack of rainfall can be a mechanical barrier to moths for emergence (Alverson et al. 1984). Rainfall eases this hurdle letting moths come out with limited effort of energy. In this case, individuals should have ended diapause development and accomplished most or all post-diapause development beforehand, while rainfall acts as a "releaser" (Denlinger 1986) thereafter. But, this phenomenon is unlikely to be true in our case. If it had been true in our case, moths would have emerged earlier in year with higher rainfall. But, our result is opposite to this. Second, rainfall may have acted as a behavioural cue for post-diapausing development. Third, there could be an interaction of rainfall with temperature, which together may play role in diapause termination or post-diapause development or both. Powell (1974) was able to break the diapause of few moths through artificial watering.

This is to keep in mind, even if emergence is a behavioural phenomenon, all individuals do not respond to rainfall immediately. In other words, individuals do not emerge at a time even if the soil is soft enough for emergence. The response to rainfall or softness of soil varies among individuals according to their relative physiological and behavioural difference, as well as, difference in their position in the soil profile. The range between the emergence of first and last individual in a year should be related to these factors as well.

Despite the existence of hosts of hypotheses about rainfall, we suppose the action here is likely played by soil humidity that should slow down the development of the pupae. This aspect remains unexplained in its mechanism but can be ascribed to the preference of the moth for the dry sites, where it has better survival as a pupa (Markalas 1989) and as a young larva (Pimentel et al. 2011). Though speculative, we would like to broaden our view about rainfall. Say, the first rainfall of the year acts as a signal threshold for post diapause development discussed hereinbefore. Instead of responding immediately to the phase of first rain, they keep waiting to assess the condition outside. They should have considered the aftermath of emergence in an intense rainy condition relative to moderate rainy condition. Intense rainy condition should be hazardous for the survival of their egg bathes and hatched larvae thereafter. Therefore, in any case, they try to avoid intense rainfall and wait for a better drier condition that in the long run will support their progenies.

Our discussion so far clarifies fairly the important role of temperature and rainfall in the emergence time of pine processionary moth in Venosta. Next step of the work would be to incorporate temperature and rainfall in a model for the prediction of the emergence time in a number of sites, and its validation with field data from pheromone traps.

4.3 Relationship between extended diapause in pine processionary moth and general theory

A hypothesis has to travel a long way of rigorous experimental evaluation before it is accepted as a theory. Same way, generalizing with any existing theory will not be very practical in case of pine processionary in our study site. We need to be very careful before we draw any conclusion. Proper ecological study on extended diapause of pine processionary moth is so far unavailable due to the problem of long time, budget and effort requiring in conducting experiment. In addition, this species is not easy to handle because of its obnoxious urtication effect (Battisti et al. 2011, Rodriguez-Mahillo et al. 2012). To the best of our knowledge, this is the first attempt of documenting the ecology of extended diapause in a natural population of the pine processionary moth. However, we are curious to discuss few possible aspects of being included in general theory regarding extended diapause.

Let's see which theory based on genetics fits our case. One theory suggests that the variability in diapause duration in a given population emerges due to the existence of several distinct genotypes in the population with respect to the diapause length, not plasticity. This case of genetic polymorphism is unlikely in our case, because when pine processionary individuals are taken into laboratory condition, almost all of them emerge due probably to temperature. Very small number (2-5%) goes to extended diapause (Démolin 1990, unpublished data).

Phenotypic plasticity makes variability in nature of diapause in pine processionary possible. Theoretically, there are two possibilities in this case. If the decision of the nature of diapause depends on any predictable environmental cue, it is a predictive plasticity (synonymous to conditional polyphenism). On the other hand, if nature of diapause is not decisive on the basis of environment, that is randomness of environmental noise does not allow proper prediction of future event, it would be a coin-flipping plasticity (Cooper and Kaplan 1982). However, since coin-flipping plasticity does not always correspond to experimental data, a modification comes out of the observation. Menu and Debouzie (1993) wrote an excellent entry on the strategy of prolonged diapause in chestnut weevil (*Curculio elephas*). They hypothesized a "mixed coin-flipping strategy", dubbed synonymously as "adaptive coin-flipping variation" or "intra-genotypic strategy mixing" or "stochastic polyphenism", in that species. There are different hypotheses on how this mixed coin-flipping strategy works. Walker (1986) assumed genotypes will be selected for their capability to flip the coin in response to unpredictable environment, and there would be fixed pattern of diapause duration (e.g., 60% simple diapause, 20% of two-year diapause, 10% of three year diapause and so on...). In contrast, although in agreement with the first statement of Walker, Menu and Debouzie (1993) disagree with the second statement that there should be a fixed pattern.

So, what tactics should pine processionary moth follow? Frequent demographic explosion is a common characteristic of the species following predictive phenotypic plasticity (Basedow 1977, Annila 1982, Eichhorn 1982). In addition, their relative growth rate [calculated as the logarithmic range {Ln=(N_{max}/N_{min})} of population size N] is higher (for example, 4.5 for *Cydia strobilella* over 12 years in Annila 1982) than that of following mixed coin-flipping strategy (1.73 for *Curculio elephas* over 12 years).

When we consider our population of Venosta, which is a range expanding marginal population, we see a sudden eruptive form in 1999. Although the population has been suppressed through insecticide and natural enemy, an eruption of pest is expected in the future. In addition, our pheromone trap data shows fairly higher relative growth rate (around 3.81). Although both characteristics should include our population into a predictive phenotypic plasticity, we refuse to accept so. The issue of predictive plasticity could be true in the core areas of pine processionary moth; however, the life history of the population and related hypothetical discussion encourage us to hypothesize a mixed coin-flipping strategy in the expanding population of Venosta.

4.3.1 Hanski's legacy

Diapause determination can be controlled both by parents or their offspring (Danks 1987). Hanski (1988) assumed that the possibility of extended diapause is likely when (i) parents are in control of diapause determination if it is density-independent, (ii) either or both parents and their offspring are in control of diapause if it is density-dependent. In addition, if the condition (i) is true, all parents should produce same mixture (which gives highest fitness in a particular environment) of non-diapausing and superdiapausing offspring. In contrast, if the diapause is density-dependent, different females may have different mixture of both diapausing natures. The mixture value should emanate equal fitness of breeding females (if they are in control) and offspring (if they controls diapause). Therefore, between-females differences in proportion of both diapausing nature offspring suggest density-independence while no differences suggest either of density-independence and density-dependence diapause. He in turns assumed that forest insects with occasional outbreaks should necessarily be density-dependent prolonged diapause.

Hanski's assumptions about prolonged diapause reflect organism-centred view of evolution rather than gene-centred ones (Dawkins 1976). Although strategy-mixing genotype can cause some individuals bear inferior phenotype, however if viewed from the level of replicator (Dawkins 1982), it incurs long-term advantage of the genotype.

We remind the reader again that characteristics in the core population and expanding population of pine processionary may defer in various ways. We saw in expanding Venosta population that outbreak started mostly due to abiotic factor rather than density dependent factor. Hanski's observation of species having occasional outbreak following density-dependent prolonged diapause strategy might be true. Population in the core areas might have the dependency on density and phenology could be predictable. We don't agree, however, with the view of Hanski that only parents should be in control of diapause in the species following density-independent strategy. Our observation, result and discussion reveal the possibility of flipping the decision while needed, which can be accomplished by the offspring in action.

Coin flipping plasticity is assumed to be important in the marginal populations (Waldbauer 1978). Furthermore, prolonged diapause in stochastic environment can be a factor increasing invasion speed (Mahdjoub and Menu 2008). Although dispersal is an alternative for diapause, nature will favor the prolonged diapause if the environmental fluctuation is well synchronized across the population (Gadgil 1971, Hanski 1988). In addition to adopting prolonged diapause, the moth can fly though minimally. Less capability of fly should be overcome by its prolonged diapause strategy. However, both capability of fly and prolonged diapause in the life history of pine processionary moth have made it an unbelievably successful species.

Our discussion regarding strategy of prolonged diapause in pine processionary somewhat explains why this species has been so fabulously successful in a wide existing range as well as in invading at high-speed to a new range, even in harsh stochastic condition.

4.4 Body size and energetics in pine processionary moth

Insects are conscious about their energy reserve status (Hahn and Denlinger 2007, 2011). Enough reserve during preparatory period should help them decide whether to enter diapause or not. On the other hand, low storage should make them terminate the diapause.

Energy reserve is more important in pine processionary moth because they enter diapause during pupal stage where there is no chance of renovating vigour through feeding. They need supply of stored energy during burying in pupation site; throughout diapause development though at suppressed level; for post-diapause development; for splitting the soil during emergence; and most importantly for post emergence flight and reproduction. In addition, summer diapause in this species should be more energy consumptive due to increased experience of temperature. Individuals of pine processionary moth that do not sequester enough energy reserve may experience three possible fates: avert entrance of diapause; if entered they die; or terminate diapause prematurely when reserve level is critically low (Hahn and Denlinger 2011).

Nutrient reserve is generally higher in the individuals with higher body mass. Therefore, individuals with larger body size should have higher potentiality to go for diapause. However, body size might not always be apparent in all cases. In addition, both reserve and body size can be diapause independent thus having no separation in these traits between diapausing and non-diapausing individuals (Saunders 1997, Siegert 1986).

Drawing any concrete conclusion from our biased (biased in the sense, we took individuals from the cage where feeding could have had a negative impact on later development) method of size measurement is dubious. Despite the fact, pine processionary might have, according to our preliminary result, increased body size thus storage of metabolic reserves as part of its diapause strategy. This strategy may have negative impact on the survival. For instance, extended period in feeding for accumulating increased energy reserve increases the risk of being attacked by the natural enemies (Masaki 1977). However, as increased body size in diapausing individuals may result in increased fecundity (Fantinou, Perdikis, and Zota 2004, Sáringer and Szentkirályi 1980, Wang et al. 2006), the risk of being eaten might get offset by the conceivable profit in pine processionary moth. Further ecological and physiological studies may reveal unknown mysteries in this species.

4.5 Climate change and extended diapause in pine processionary

Can increased temperature due to climate change reduce the intensity of prolonged diapause in pine processionary moth? Before discussing this issue we need to take a look at the meteorological data. However, the issue should be considered globally, regionally and locally. An increasing trend of warming especially in summer is apparent all over the Mediterranean basin. On the other hand, an overall declining trend in precipitation has been found in the Mediterranean region except for northern Mediterranean Alps where mean wet days is on decrease but total rainfall has a little bit increasing trend due to high intensity of precipitation (Brunetti et al. 2004, Brunetti, Maugeri, and Nanni 2001, Gao, Pal, and Giorgi 2006, Giorgi and Lionello 2008, Sánchez et al. 2004).

Projecting the impact of climate change in the prolonged diapause of pine processionary in Venosta (or to say Alps region) is challenging mostly due to the anomalous pattern of local rainfall. Here, we will discuss few aspects of probable effect.

Rainfall has been reported to prevent termination of diapause thus prolonging it (Geering and Baillie 1954) in tropical diapausing insect. Pine processionary could have inherited the genetic basis of diapause from its tropical diapausing ancestor. If the rainfall works as a factor of prolongation of diapause in this species, an increase in precipitation should increase the intensity of its diapause. On the other hand, if rainfall works as a factor of removal of soil mechanical barrier as per one of the assumptions, increased precipitation over the area should reduce this obstacle thus reducing energy cost and rate of mortality. We still don't know which one is the case in our Venosta population.

However, although the total precipitation has increased in this area, total wet day has decreased resulting in higher proportion of days without rain. In addition, comparatively higher rainfall has been produced from increased rainfall in winter (Brunetti, Maugeri, and Nanni 2001). We need to settle through experiment if sporadic extreme nature of rainfall works for or against intensity of diapause in pine processionary.

If the rainfall trend turns out to be negative for extended diapause of the species, the situation will be further deteriorated by the corrosive role of increased temperature. On the other hand, if the future rainfall pattern yields positive response for intense superpause, it will be neutralized by the interplay between rainfall and temperature. Whatever happens, we assume enhanced temperature will dominate over the action of rainfall. Considering this assumption we may reach a preliminary conclusion.

Probability of reduced intensity of superpause is likely in the previous range (e.g., comparatively lower elevation or latitude) of pine processionary in a changing climate. However, since increased temperature is shifting the range of the species towards north (Battisti et al. 2006, Battisti et al. 2005), they should not have problem in extending diapause intensity as they did before and are doing now due to similar scale of temperature in action in the north.

5 Reference

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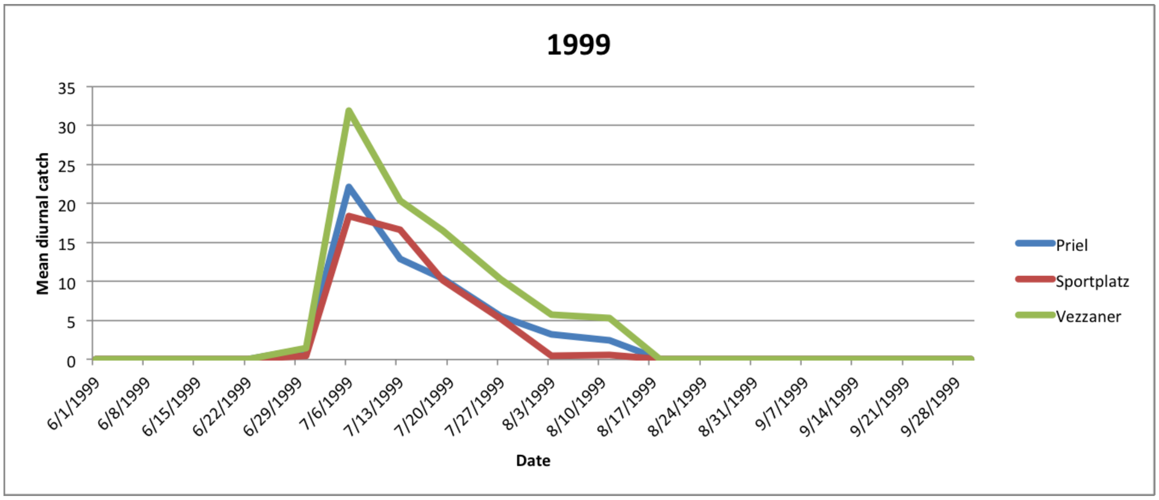
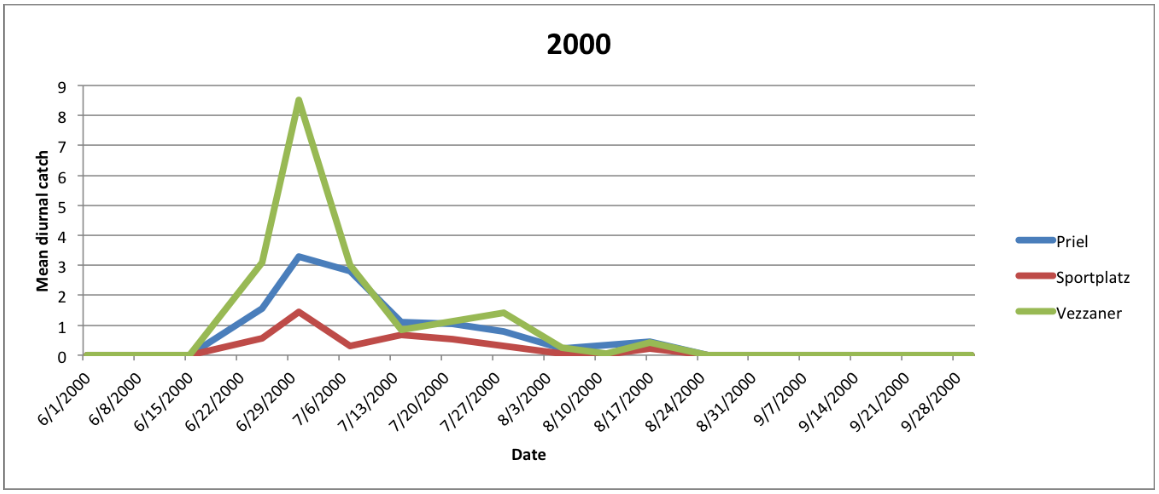
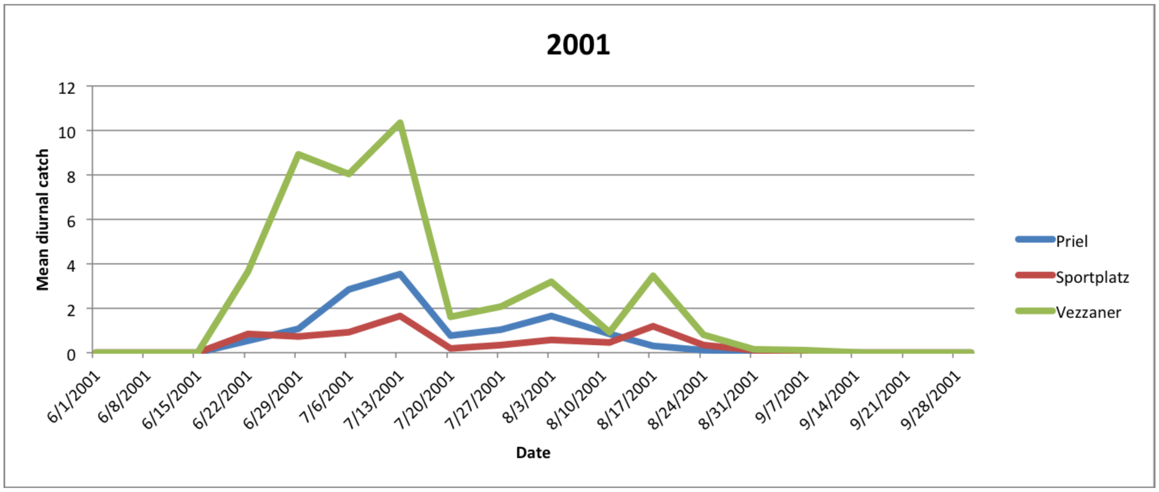
6 ANNEXES

6.1 ANNEX 1: Mean daily catch in the pheromone traps each year



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Mean diurnal catch
0
2
4
6
8
10
12
6/1/2001
6/8/2001
6/15/2001
6/22/2001
6/29/2001
7/6/2001
7/13/2001
7/20/2001
7/27/2001
8/3/2001
8/10/2001
8/17/2001
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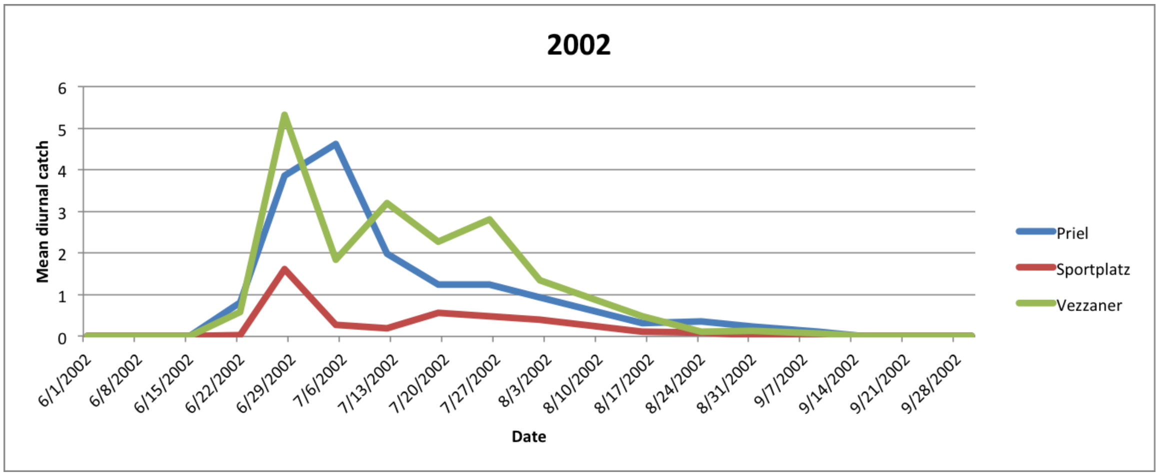
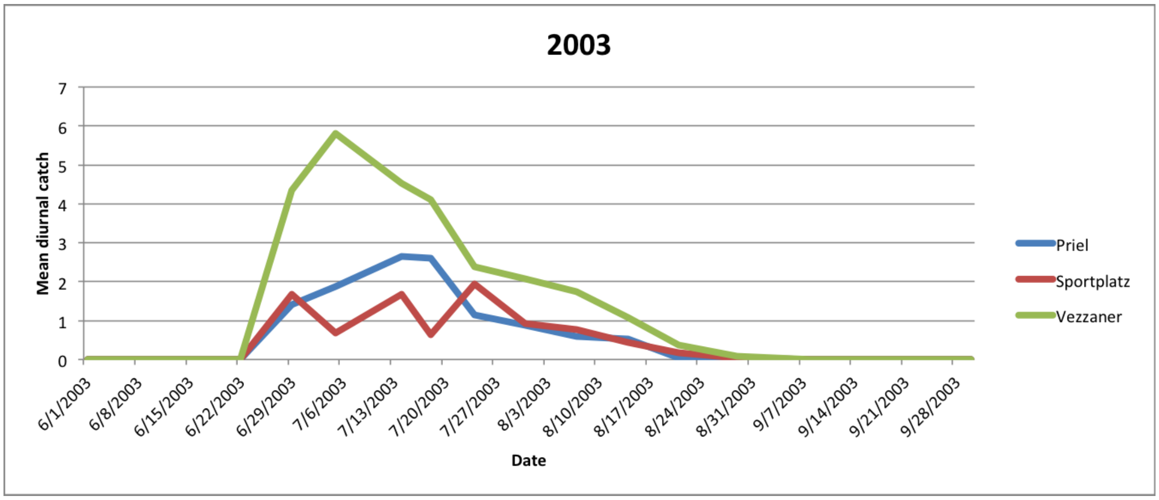
9/14/2001
9/21/2001
9/28/2001
Date
Priel
Sportplatz
Vezzaner


2002
Mean diurnal catch
0
1
2
3
4
5
6
6/1/2002
6/8/2002
6/15/2002
6/22/2002
6/29/2002
7/6/2002
7/13/2002
7/20/2002
7/27/2002
8/3/2002
8/10/2002
8/17/2002
8/24/2002
8/31/2002
9/7/2002
9/14/2002
9/21/2002
9/28/2002
Date
Priel
Sportplatz
Vezzaner


2003
Mean diurnal catch
0
1
2
3
4
5
6
7
6/1/2003
6/8/2003
6/15/2003
6/22/2003
6/29/2003
7/6/2003
7/13/2003
7/20/2003
7/27/2003
8/3/2003
8/10/2003
8/17/2003
8/24/2003
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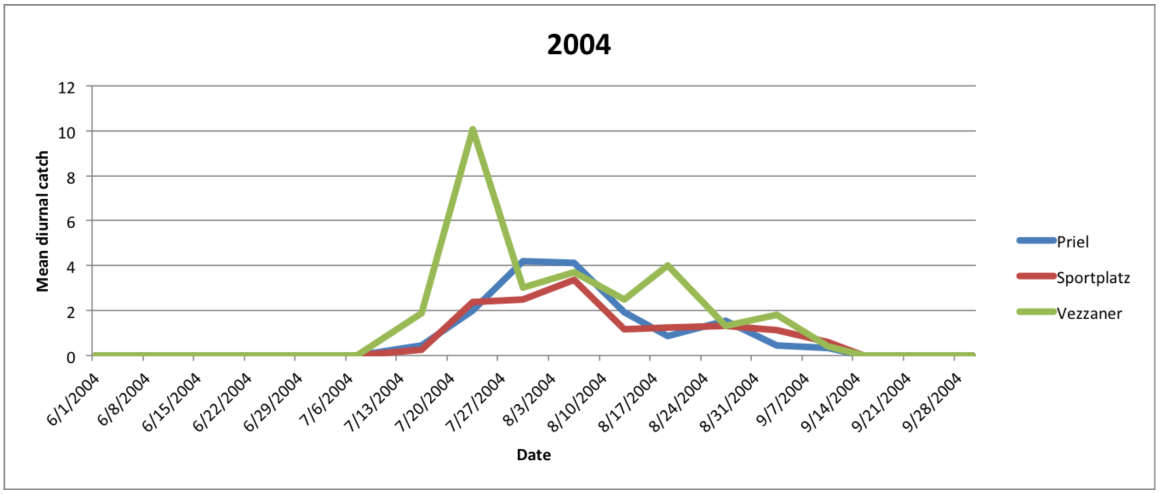
9/7/2003
9/14/2003
9/21/2003
9/28/2003
Date
Priel
Sportplatz
Vezzaner


2004
Mean diurnal catch
12
10
8
6
4
2
0
6/1/2004
6/8/2004
6/15/2004
6/22/2004
6/29/2004
7/6/2004
7/13/2004
7/20/2004
7/27/2004
8/3/2004
8/10/2004
8/17/2004
8/24/2004
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9/7/2004
9/14/2004
9/21/2004
9/28/2004
Date
Priel
Sportplatz
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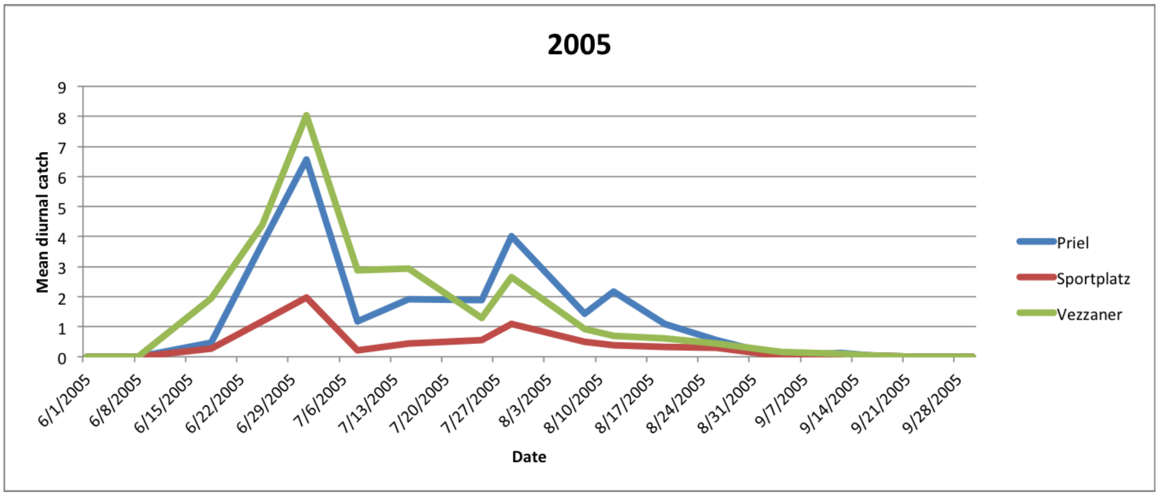
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Mean diurnal catch
9
8
7
6
5
4
3
2
1
0
6/1/2005
6/8/2005
6/15/2005
6/22/2005
6/29/2005
7/6/2005
7/13/2005
7/20/2005
7/27/2005
8/3/2005
8/10/2005
8/17/2005
8/24/2005
8/31/2005
9/7/2005
9/14/2005
9/21/2005
9/28/2005
Date
Priel
Sportplatz
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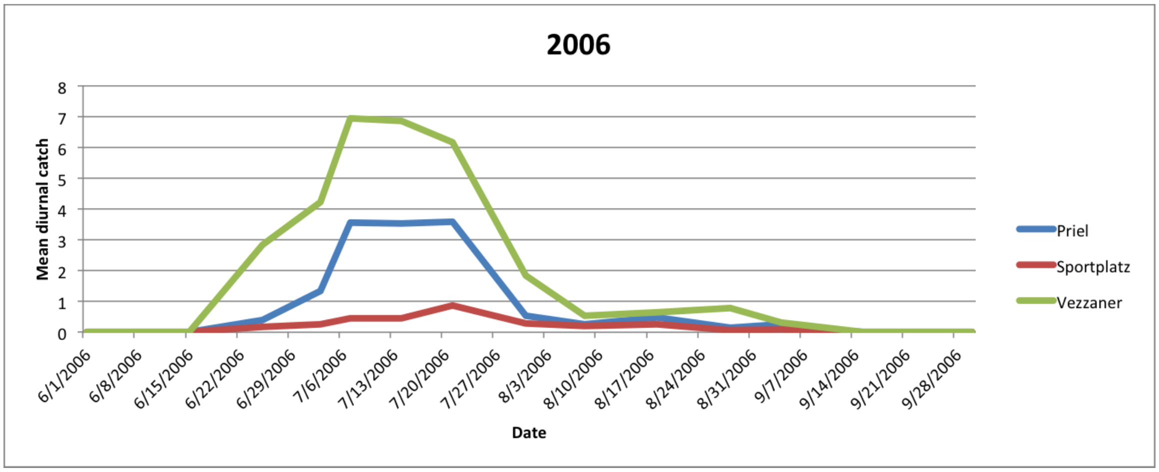
2006
Mean diurnal catch
8
7
6
5
4
3
2
1
0
6/1/2006
6/8/2006
6/15/2006
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6/29/2006
7/6/2006
7/13/2006
7/20/2006
7/27/2006
8/3/2006
8/10/2006
8/17/2006
8/24/2006
8/31/2006
9/7/2006
9/14/2006
9/21/2006
9/28/2006
Date
Priel
Sportplatz
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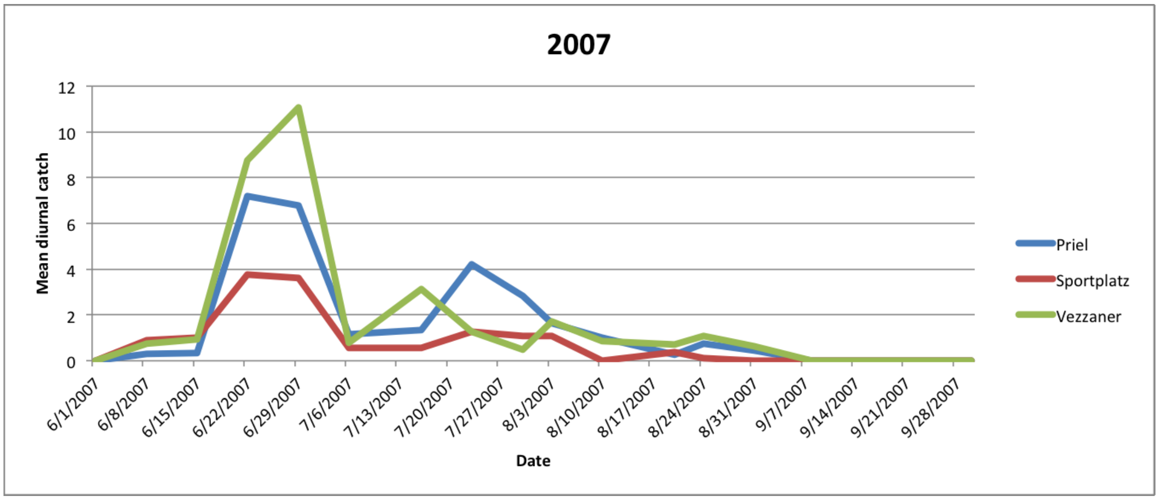
2007
Mean diurnal catch
12
10
8
6
4
2
0
6/1/2007
6/8/2007
6/15/2007
6/22/2007
6/29/2007
7/6/2007
7/13/2007
7/20/2007
7/27/2007
8/3/2007
8/10/2007
8/17/2007
8/24/2007
8/31/2007
9/7/2007
9/14/2007
9/21/2007
9/28/2007
Date
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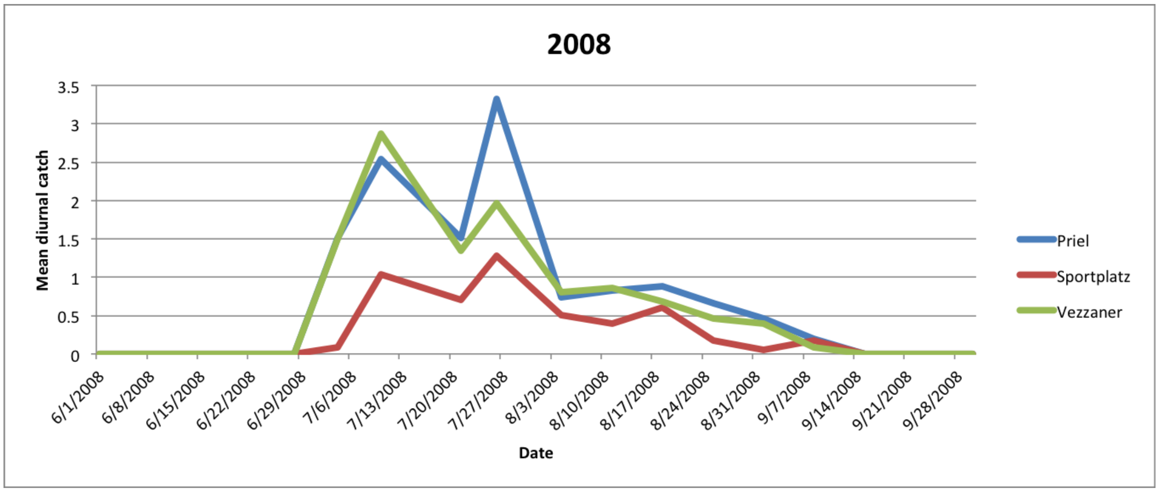
2008
Mean diurnal catch
3.5
3
2.5
2
1.5
1
0.5
0
6/1/2008
6/8/2008
6/15/2008
6/22/2008
6/29/2008
7/6/2008
7/13/2008
7/20/2008
7/27/2008
8/3/2008
8/10/2008
8/17/2008
8/24/2008
8/31/2008
9/7/2008
9/14/2008
9/21/2008
9/28/2008
Date
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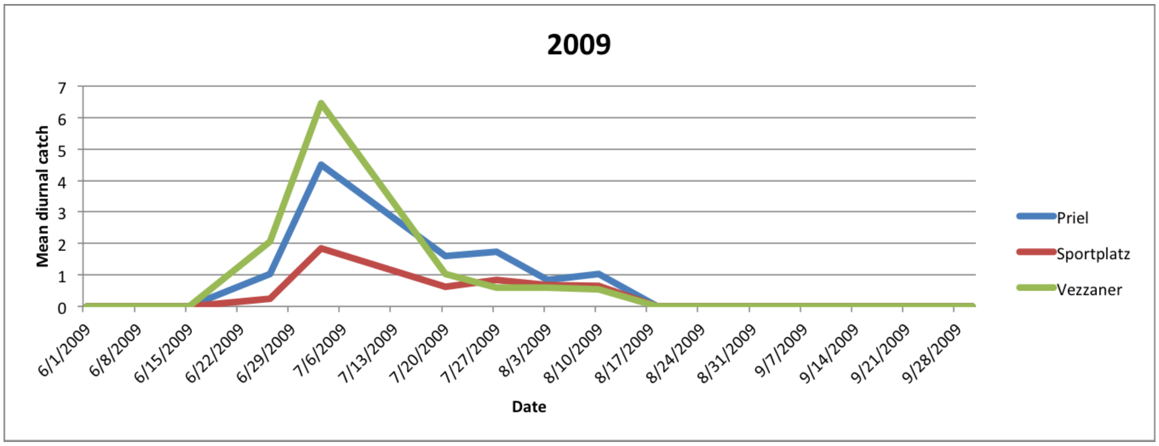
2009
Mean diurnal catch
7
6
5
4
3
2
1
0
6/1/2009
6/8/2009
6/15/2009
6/22/2009
6/29/2009
7/6/2009
7/13/2009
7/20/2009
7/27/2009
8/3/2009
8/10/2009
8/17/2009
8/24/2009
8/31/2009
9/7/2009
9/14/2009
9/21/2009
9/28/2009
Date
Priel
Sportplatz
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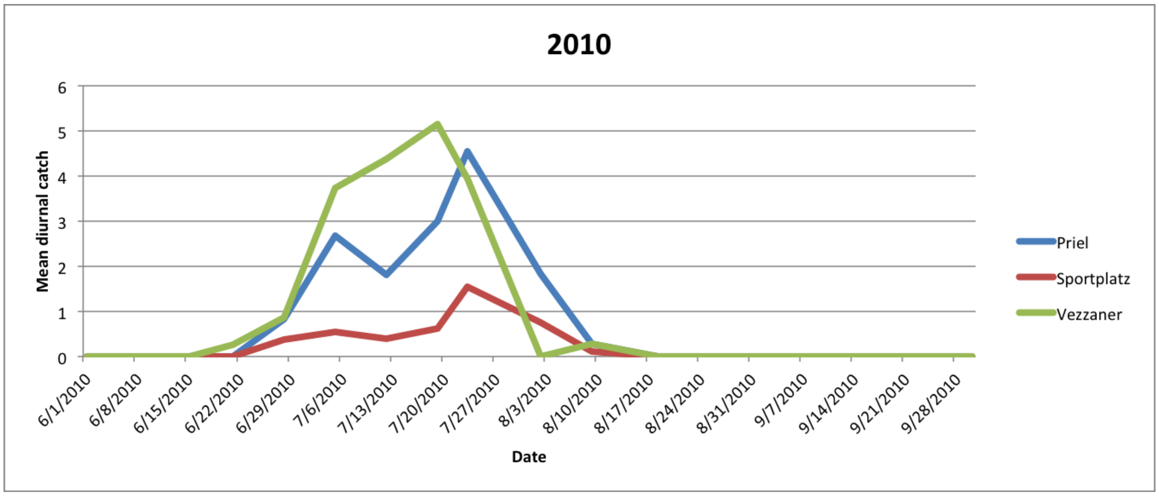
2010
Mean diurnal catch
6
5
4
3
2
1
0
6/1/2010
6/8/2010
6/15/2010
6/22/2010
6/29/2010
7/6/2010
7/13/2010
7/20/2010
7/27/2010
8/3/2010
8/10/2010
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9/28/2010
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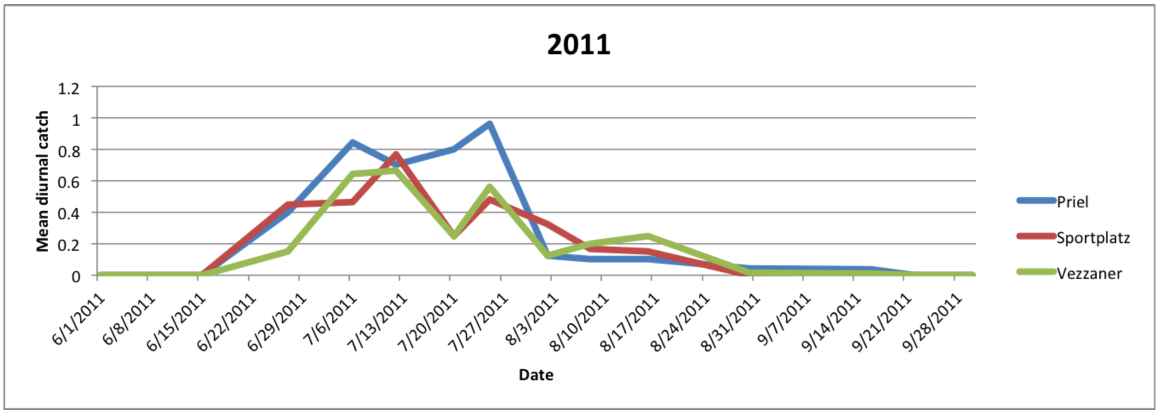
Vezzaner


2011
Mean diurnal catch
1.2
1
0.8
0.6
0.4
0.2
0
6/1/2011
6/8/2011
6/15/2011
6/22/2011
6/29/2011
7/6/2011
7/13/2011
7/20/2011
7/27/2011
8/3/2011
8/10/2011
8/17/2011
8/24/2011
8/31/2011
9/7/2011
9/14/2011
9/21/2011
9/28/2011
Date
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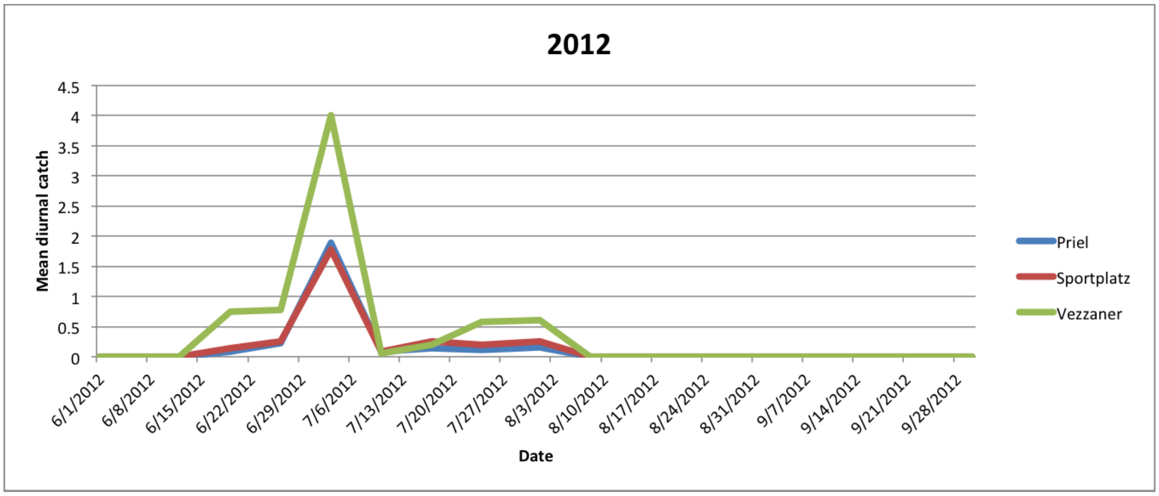
2012
Mean diurnal catch
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
6/1/2012
6/8/2012
6/15/2012
6/22/2012
6/29/2012
7/6/2012
7/13/2012
7/20/2012
7/27/2012
8/3/2012
8/10/2012
8/17/2012
8/24/2012
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9/7/2012
9/14/2012
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9/28/2012
Date
Priel
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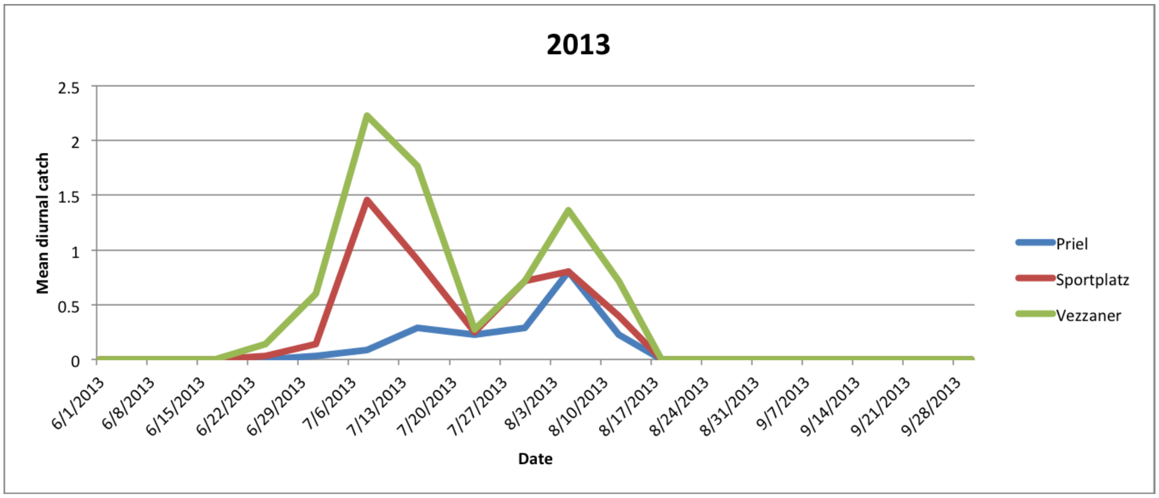
Vezzaner


2013
Mean diurnal catch
2.5
2
1.5
1
0.5
0
6/1/2013
6/8/2013
6/15/2013
6/22/2013
6/29/2013
7/6/2013
7/13/2013
7/20/2013
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Date
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Vezzaner

6.2 ANNEX 2: Partial translation and discussion of Démolin's paper on diapause

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DEMOLIN, G 1990: Réflexions générales sur la diapause et les diapauses renforcées chez la processionnaire du pin, Thaumetopoea pityocampa Schiff.. Exposé au Colloque I.N.R.A. Cycles saisonniers chez les invertébrés. Dourdan, février 90.

In his paper, Demolin simply discussed the outcome of experiments he gathered throughout his research period with Pine Processionary. It is better not just to translate his words into English, rather to capture the main ideas he wanted to put. Therefore, we will translate few important paragraphs and tables that will help us generate our own ideas. Our remarks will be put right after the relevant paragraphs. Sometimes we have mingled the literary translation and our ideas together.

Adults always emerge from their common sense on a good precise date so that their offspring possibly escape: 1) Too high summer temperatures (>30° C) that generate subsequent death of early instar larvae. 2) Too low winter temperature so that the larvae can reach a sufficient advanced stage in order that the colony can resist better the cold (lethal temperature from -10 to -17° C depending on the colonies) by building a more efficient tent.

Knowledge of a large part of the climatic requirements of the species, mainly addressed by study of social behavior, allowed us to establish, 1) A theoretical chart that schematizes all the possible cycle of this insect distribution throughout the Mediterranean. 2) Define its potential distribution area.

Types of cycles found are:

1) A and B: The populations belonging to the southern zone, producing emergence from mid-July to mid-August, and up to the end of September and October in the southern countries of the Mediterranean Basin (Israel, Lebanon etc.)

2) B and C: The populations belonging to higher elevation, emergence taking place in the early July to the mid-August (e.g., Ventoux) and for the mountains of southern Mediterranean Basin (Algeria, Morocco, Lebanon etc.).

3) C, D and E: The populations of very high elevation where emergences are very early, beginning generally at the end of June (In Corsica D and E) and oceanic zones (e.g., Landes B C D).

The period of flight of populations is virtually stable from year to year. It can extend over three to four weeks, in the month of June (in Landes) and in early October (in Israel). The "timing" is a technical word print that we used to specify the average date of these periods.

The “key period” of biotope, which corresponds to the lifting of the diapause and which precedes 40 days (at 20°C) from the period of emergence of adults, is registered in the “maternal genetic pattern” (founding factor?) and would already be predetermined in the oocyte. In fact we consider the resumption of development ("key date" for an individual) at the beginning of the formation of the adult who materialized, performing dissections of pupae, 1) by the early development of genital tracts, male or female, are already visible on the caterpillars, and 2) by the simultaneous introduction of the first duct in ovarian oocytes.

In this key period, the individual starts oogenesis (formation of sex cells). This happens not only to the non-diapausing but also to the extended diapausing pupae. But the individuals with extended diapause will remove the oocytes immediately after formation. These oocytes are changed to ‘yellow bodies’ that remain visible at the base of the ovaries. It could be possible to see this yellow stuffs if we dissect the extended diapausing individuals. This is reported also in the paper of BILIOTTI et al. (1954) without additional information. (What about the males? oocytes are produced only in females. Demolin said nothing about it. We can see if there is anything distinguishing in the males) .

Remarks:

According to Demolin, both non-diapausing and prolonged diapausing individuals resume their activity after the obligatory diapause, from where non-diapausing individuals emerge and individuals with extended diapause go to prolonged diapause. Extending individuals stops their activities right after the degeneration of oocytes in the ovaries. This remark supports the "late-switch" hypothesis over "early-switch" hypothesis (see Soula and Menu 2005). We can test this hypothesis with our model.

Our work could be to develop a method to identify the "key period" in female through our respirometry test. On the other hand, the measurement of respiratory activity can be measured in the male as well to see if there is any fluctuation in the activity. We assume the assumption of Demolin is true and possibility to find this period in female is certain, but finding the same period in the males would be totally a new finding.

Our task would be to sex them, weigh them, and record the respiration continuously.

If you put the pupae in high temperature (30 degree), you can modify the emergence time in one-year cycle populations. In that case, the emerged individuals will be weak and possibility of mortality inside the pupae will be higher due to temperature stress.

If you put the higher temperature during the diapause time of pupae, it will postpone the emergence. That is, higher temperature will slow down the pupal development and the individuals will go into prolonged diapause. It has an ecological meaning. If the summer is hot any year, the individuals will not *emerge in the time generally they come out, rather they will extend their emergence. But, that might not change the extended diapause. This is very important in the south of the Mediterranean basin.*

Acquisition of biannual or multiannual cycle:

1) When working with one-year cycle population:

a. If you put the mature larvae or pupae into low temperature (4-6 degree), they stay as they are, that is, they stop development.

b. If you put them into 20 degrees after the key period, that is, after the activation, you will have the emergence only after 40 days (which is earlier than the normal time). *So, if you want to have earlier emergence of the adults, you can put them into 20 degrees during the key period. Our colleague Laura did the same experiment. She took the larvae and reared them in more or less room temperature, and she didn't have any individual with extended diapause. Therefore, it is practical to manipulate the emergence of individuals through the treatment of higher temperature.*

2) Working with relatively low temperature (12 to 14 degree):

If you put them into this temperature before the key period, the development slows down. Due to this slowness, a fungal pathogen may grow (*Beauveria bassiana*), and ultimately, the insect individuals extend their emergence to next year or more. So, this relatively low temperature makes them go to extended diapause. In the experiment, Demolin took the larvae before key period, and kept a control into 20 degree of which almost 90 percent are annual.

Then, every 4 days, he took a group and put them into 20 degrees. Ultimately, the group that has been put to 12 degrees for longer, he had only 2-year life cycle. The shorter he put them into this temperature; the more the one-year life cycle prevailed. *Cold spell prolonged the larvae to pupate. So, the longer is the cold period, the later would be the pupation, the higher would be the diapause.*

3) What if you put the larvae into different degree of shade?

Demolin took the processionary larvae and put them in different shade condition: few in the full sunlight, few in semi-shade, few in full shade and few in the underground. The result is shown hereunder as table:

	Control	**Full sunlight**			**Semi-shade**			**Full shade**			**Underground**
		AI	*AII*	*AIII*	*BI*	*BII*	*BIII*	*CI*	*CII*	*CIII*	*D*
Cycle 1	86.7	99.8	98.2	98.7	96.5	97	99.4	65.5	16.7	58.5	19.6
Cycle 2	13.3	0.2	1.8	1.3	3.5	3	0.6	36.5	36.5	30.5	80.4
Cycle 3	0	0	0	0	0	0	0	0	0	11	0

Remark:

The result indicates there is not that much difference between full sunlight and semi-shading. But these two treatments significantly differ with full-shade treatment. Therefore, shading has an effect on extending diapause. It explains why larvae go to sun and pupate there. If we want to do experiment, we should be careful about the shading environment, because that could modify the situation.

Translocation experiment:

He took eggs from type A and D and translocated them. In his case, he took

1. Local population of Ventoux (A type) and raised locally
2. Local population of Corsica (D type) and raised locally
3. Local population of Corsica and raised in Vantoux.

A. Experiment results in the field condition: Translocation from type D to AB, reduced the percentage of prolonged diapause

Cycles	**1**	**2**	**3**	**4**
1	81.7%	18.3%	0%	0%
2	7.8%	90.2%	2%	0%
3	0%	54%	12%	34%

B. Experiment results in the laboratory condition: Taking the both type into the laboratory condition significantly increases the rearing time and reduces the extended diapause increasing the one or two year life cycle.

Cycles	**1**	**2**	**3**	**4**
Ventoux population	98%	2%	0%	0%
Corsica population	45%	53%	2%	0%

An interesting thing he pointed out in his paper on parasitoid of ppm. Without any data he said there was a parasitoid in Corsica, which goes into extended diapause synchronizing with the diapausing individuals of pine processionary.

Is it possible to change the period of extended diapause once the extended diapause already has been induced?

What is the effect of temperature applied immediately after pupation in the pluriannual (D) type?

1. Temperature of 12-14 ° C applied on pupae of Corsica shortly after pupation:

Corsica population generally has a two-year life cycle. Once they have been treated with this temperature, the percentage of extended diapause increased.

2. Temperature of 29-31 °C applied on pupae of Corsica shortly after pupation: This treatment increased the percentage of general 2-year cycle and reduced the percentage of extended diapause. Even few one-year cycle's individual showed up.

Remark:

From his paper, we see that it is possible for one type of population to switch into another due to the variation in temperature. For example, in the continuous monitoring of Ventoux (850 m altitude) population on-site, he observed that all but 1977 and 1978 population are of type B, but those two years switched to D and E type, which was correlated with cold spring. Generally it should have followed pattern B all the year, but the trend did change.

Although we don't know how he did his experiment, but we clearly have some intriguing ideas to proceed on. So far we have seen, two very important factor came out from the discussion are **"temperature"** and **"key period".** If his ideas are true, it will have significant importance in the understanding of ecology of pine processionary. To test his ideas, we can try to find out answers to few questions.

First, we can look at the peaks of emergence (from the pheromone trap), which indicates the relative contribution to emergence between annual and extended diapausing individuals, and see if there is any climatic factor (especially temperature) correlated to it. In this case, we can predict the likely outcome of emergence in a retrospective way. But, to be able to do this, we should have an efficient method to identify the "key period" in the field. Once we are satisfied with the result, then we can hypothesize either of these two:

1. There is a continuous response to temperature,
2. There is a threshold level of temperature for the response.

So, ultimately one should be able to measure the soil temperature during the "key period" as a way to predict the possible outcome in the next year.
We can test different thing:

1. *Short-term:*

1) We have around 100 extended diapausing pupae from the 2013 cohort. We initially can test the matter of "key period" and test the "late switch" hypothesis (repeated diapauses rather than initially-programmed prolonged diapause) through the measurement of CO_2 and temperature emission. For this, we already have efficient equipment.

So, we expect:

i. Low gas release now and low temperature,
ii. Increase of both during the key period,
iii. Maintenance or increase of gas release and temperature for the pupae going to produce adults in 2014, regression to low values for the others -> late-switch confirmation.

The idea is to plan measures every week in a continuous way for 2-3 days.

2) Interpopulation variability of PD frequency. Intrapopulation variability with density of nests per tree and larvae per bag available as covariates. Influence of pupae weight and gender on intrapopulation variability can also be examined at the laboratory.

3) Field (cage)/field (bags)/laboratory differences? For Marcillac: comparison of PD frequency and emergence date between field samples (cages) and bags translocated to Orleans.

4) First trials on the efficiency/relevance of the sampling methods and respirometry equipments. Depending on results, benefits of the Ecopièges compared to cages can be assessed.

2. Long-term tests:

1) Effects of burying depth in the soil (related to temperature experienced by pupae, related to body size (the bigger the individual the deep inside they go) on average PD frequency and duration.

2) Effects of soil properties (loose *vs.* hard, aeration level, density, moisture level, vegetal cover, *etc.*).

3) Density inside trees/nests/soil.

4) Climatic conditions, is pupae the sensitive instar? Is it the only sensitive instar (likely if the "late switch" is confirmed)?

5) Date of processions within the procession period?

6) Verification of delay in the emergence peak after regular vs. prolonged diapause.

7) Differences in life-histories between individuals from regular vs. prolonged diapause?