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台灣北部暫時性池塘大型鰓足類的分佈與孵化策略

Large branchiopod’s distribution and hatching strategies in a freshwater temporary pool in northern Taiwan

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摘要

暫時性水池的積水相變動隨機且不穩定，時常在積水與乾涸之間頻繁交替，棲息於其內的大型鰓足類已演化出特殊的生活史，產下能忍受乾旱等極端環境的休眠卵以適應暫時性水池。休眠卵非同步孵化的特性，使得部份未孵化的休眠卵會逐漸累積在底質中形成休眠卵庫，持續休眠直到下一個積水期的到來。能否維持休眠卵庫是大型鰓足類在暫時性水池存續的關鍵。在空間上，牠們必須將休眠卵產在最理想的區域—水池邊緣，以確保休眠卵能在池水水量較充沛、積水期較長時孵化，而在時間上，休眠卵除了得在一個積水期內的適當時間孵化以避免生殖失敗，也應該適當分配孵化量至不同積水期，減少無效孵化所造成的休眠卵庫損失。因此，本論文分別從空間與時間面向，瞭解鵲沼枝額蟲、真湖蚌蟲與貓眼蚌蟲等三種共域於向天池的大型鰓足類，是否達成理想的休眠卵邊緣分佈與是否表現出理想的孵化率，以探討各物種的生存策略。

論文第一章關注大型鰓足類成體的空間分佈模式。涵蓋四個積水期的逐日穿越線垂直拖網採樣結果顯示，向天池的三種大型鰓足類中，只有鵲沼枝額蟲表現出穩定聚集於水域邊緣的現象，然而其在向天池的分佈熱區卻與貓眼蚌蟲類似，都集中在中心三處最深的小凹洞區域；真湖蚌蟲的熱區則相對廣布，但是並不均勻，較偏向低植被覆蓋的步道區域。此分佈模式可能與各物種的性成熟時間和積水範圍的日漸縮減有關，雖然鵲沼枝額蟲會主動聚集在水域邊緣，但是最後卻與更晚成熟的貓眼蚌蟲同樣侷限在向天池三個水坑中心附近的區域，而較早成熟的真湖蚌蟲在棲地利用上限制較少，因而能分佈於更廣闊的範圍，然而其不均勻的空間分佈現象可能意味著此物種有特殊的微棲地偏好。

延續空間分佈的議題，論文第二章轉向休眠卵，估算大型鰓足類的休眠卵庫數量，以及釐清牠們在向天池內的分佈狀況。系統性採集向天池表層底質並經過辨識、計數休眠卵數後，發現貓眼蚌蟲是休眠卵庫數量上最優勢的物種，而鵲沼

枝額蟲的休眠卵庫數量接近批次生產力，表示休眠卵庫可能會達到數量上的動態平衡，而非無限制的累積。在分佈方面，鵠沼枝額蟲的休眠卵集中在向天池的中心三處水坑，真湖蚌蟲偏向於西北側，而貓眼蚌蟲則廣泛分佈，特別是池子的南側直到更邊緣的區域。這般種間各異的模式可能是由許多不同的機制導致。

一併檢視成體與休眠卵庫的分佈，發現鵠沼枝額蟲在兩者熱區的分佈模式上互相吻合，但對於真湖蚌蟲與貓眼蚌蟲而言卻並非如此，意指鵠沼枝額蟲休眠卵庫的水平分佈主要由成體的分佈模式決定，然而真湖蚌蟲與貓眼蚌蟲的休眠卵還受到休眠卵的再散佈影響。由於較晚成熟與成熟時受限的積水範圍，鵠沼枝額蟲沒有辦法將大多數休眠卵產在理想的向天池邊緣區域，但是更晚熟的貓眼蚌蟲卻可能因為休眠卵的再散佈，而得以重新傳播休眠卵到池子邊緣。整體而言，在先天發育速率與積水相造成的空間利用限制下，大型鰓足類仍舊可能間接受益於其它機制，以達成理想的休眠卵分佈模式。

就時間面向探討，論文第三章針對鵠沼枝額蟲與真湖蚌蟲的孵化物候，藉由實驗室孵化實驗，每週重複浸泡、乾燥休眠卵，並紀錄積水期內每天的孵化數量，結果顯示這兩種的孵化時間均集中在一個積水期剛開始的二到五天內，以確保孵化的個體能盡早成熟。此外，主要的孵化量都發生在早先的二到三次積水期內，而非均勻分散至往後更多的積水期；如果每批孵化的個體有較高的機率能順利成長並生殖，孵化策略上便無需太過保守，可以趁早孵化。向天池積水相的特色—與豪大雨同步發生的積水期，使得積水時通常擁有豐富的水量且能維持足夠時間，因此降低了大型鰓足類在積水期內孵化卻未能生殖的風險。

接續孵化物候，為了探究大型鰓足類的孵化策略，論文第四章試圖釐清鵠沼枝額蟲與真湖蚌蟲是否因應在向天池面臨到的長期生殖成功率變化，而表現出理想的孵化率。分別在兩種孵化的風險情境—孵化率是以每次積水期、或是以每個年度為評估基準，驗證孵化率與生殖成功率是否一致(柯恩氏假說)。結果發現鵠沼枝額蟲的孵化率在兩種情境中都與生殖成功率有顯著差異，而真湖蚌蟲的孵化率

則在後者情境中與生殖成功率吻合，意味著真湖蚌蟲的孵化策略趨於保守，以年度內的數次積水期為單位進行賭注，減少了族群生存適度在積水期間的變動幅度。鵠沼枝額蟲的孵化率較理想狀況為低，可能是缺乏理想化孵化率的選汰壓力，亦有可能肇因於先天生理限制而無法表現出理想孵化率。

綜觀這三種共域的大型鰓足類，研究較多的鵠沼枝額蟲與真湖蚌蟲可能分別依賴生命週期的不同階段以生存於向天池。鵠沼枝額蟲活動階段的特色—較大的族群量與機會性的彈性生長模式等，可能讓鵠沼枝額蟲擁有較高的生殖力，以抵銷偏低的孵化率對維持休眠卵庫的影響。其它兩種則明顯不同，真湖蚌蟲的休眠階段除了有較高的孵化率，孵化時的保守性風險分散策略可減少生殖失敗造成的休眠卵庫損失，避免族群生存適度的大幅度變動，而目前貓眼蚌蟲的生活史與孵化物候尚待孵化養殖技術突破才能進一步釐清。

關鍵字：鵠沼枝額蟲、真湖蚌蟲、貓眼蚌蟲、積水相、分佈、休眠卵庫、風險分散策略

Abstract

Temporary pools are characterized by stochastic hydroregimes, which fluctuate frequently betweeen wet and dry periods. Large branchiopods have adapted to these conditions via specific life history traits including the production of dormant eggs which accumulate in the substrate as an egg bank, remaining viable until the next hydroperiod. Maintaining the egg bank is key to long term persistence. Spatially, eggs should be placed in optimal regions to ensure hatching when there is sufficient water to complete the life cycle. Temporally, they should hatch at the optimal point within a hydroperiod to prevent reproductive failure, and in optimal fractions across hydroperiods to reduce egg bank depletion. I studied the large branchiopods in Siangtian Pond, i.e. *Branchinella kugenumaensis*, *Eulimnadia braueriana*, and *Lynceus biformis*. I explored their optimal spatial and temporal egg and adult distributions, optimal hatching rates, and discuss their life strategies.

The first chapter focuses on distributional patterns of free swimming adults. Daily transect line surveys with vertical townet samplings across four hydroperiods showed that only *B. kugenumaensis* consistently aggregated at the edge, while overall density hotspots for both *B. kugenumaensis* and *L. biformis* were concentrated centrally in the three deepest places. Even though *B. kugenumaensis* aggregated at the edge of the inundation area, generally late maturation limited the available region for it and *L. biformis* near the center. In contrast, *E. braueriana* hotspots were widespread but skewed towards less vegetated places. Early maturing *E. braueriana* suffered less restriction on habitat utilization and was able to distribute widely, although the skewing implies special microhabitat preference.

Continuing in spatial aspects, the second chapter estimated egg bank sizes and

clarified structure. Identifying and counting eggs in the superficial substrate of core areas revealed that *L. biformis* was dominant numerically. Egg bank size for *B. kugenumaensis* was close to cohort fecundity, meaning that egg bank would balance around a certain number rather than accumulate unlimitedly. Regarding spatial structure, *B. kugenumaensis* eggs were distributed in the central three deepest places, inclined to the northwest in *E. braueriana*, and spread widely towards the southern margin in *L. biformis*. Species specific patterns can be realized from multiple mechanisms.

Taking the above two chapters together, egg bank spatial patterns were coincident with adult hotspots in *B. kugenumaensis*, but not in *E. braueriana* or *L. biformis*. This indicates that horizontal structure for *B. kugenumaensis* eggs was mainly determined by adult distribution, while it was also affected by factors such as egg redispersal in *E. braueriana* and *L. biformis*. The peripherally aggregated *B. kugenumaensis* failed to distribute eggs along the basin margin due to late maturation and accordingly restricted inundation areas near the three deepest places. However, redispersal may work and redistribute eggs to the basin edge for the late maturing *L. biformis*, providing opportunity in spite of developmental limitations.

Regarding temporal aspects, the third chapter examined the hatching phenologies of *B. kugenumaensis* and *E. braueriana*. Laboratory experiments through repetitive inundations and dehydrations at weekly intervals demonstrated that hatching of both species concentrated in the second through fifth day of a hydroperiod, which could assure early maturation. Additionally, the majority of hatching fractions occurred in the first two to three inundations rather than spread across more inundations. Since the hydroregime of Siangtian Pond, i.e. synchronization between large scale precipitations and hydroperiods, can ensure abundant water and longer hydroperiods, the risk of abortive hatching could be low and thus large branchiopods have evolved an early

hatching time.

As regards hatching strategy, the fourth chapter tested if *B. kugenumaensis* and *E. braueriana* showed optimal hatching following the variation of successful reproduction (Cohen's hypothesis) under two scenarios wherein a species hatches according to a sporadic hydroperiod or a year with several hydroperiods. I compared hatching rate with evaluated successful reproduction rates based on long term life history and climate data. The two rates were different for *B. kugenumaensis* in both scenarios but were identical for *E. braueriana* in the latter scenario, suggesting that *E. braueriana* bet hatching on yearly cycles. *Eulimnadia braueriana* adopted a more conservative hatching strategy, further lowering fitness variation. The apparently unfavorable hatching rate in *B. kugenumaensis* may result from weak selection for optimization or physiological limitations.

As a whole, *B. kugenumaensis* and *E. braueriana* may rely on different stages of their life cycle to survive in Siangtian Pond. Characters of free swimming individuals such as abundant population sizes and flexible growth patterns could lead *B. kugenumaensis* to be more fecund and balance hatching performance inferiority. On the other hand, high hatching rate accompanying conservative bet hedging hatching in *E. braueriana* eggs help to reduce the depletion from abortive hatchings and avoid fitness extremes. Breakthroughs in triggering *L. biformis* to hatch under laboratory conditions will contribute to the understanding of its life history and hatching phenology in the future.

Keywords: bet hedging, *Branchinella kugenumaensis*, distribution, egg bank, *Eulimnadia braueriana*, hydroregime, *Lynceus biformis*

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Chapter 1: General introduction

Freshwater temporary pools represent one of the extreme habitats. Unlike permanent pools which hold water forever or for a significant long period (Seaman et al., 1995), temporary pools are characterized by its unstability and unpredictability. Depending on the climate regime (Williams, 1985), it may change periodically between inundation and drought (Huang et al., 2010), freezing (Daborn, 1975; Mossin, 1986; Saiah and Perrin, 1990; Mura, 2001; Zarattini and Mura, 2007), muddiness (Boudrias and Pires, 2002; Rogers et al., 2006), or extremely fluctuating temperature (Brendonck et al., 2000; Jocqué et al., 2010). Also, the water may come from various sources including rain, flooding (Eder and Hödl, 2002), melting snow, or even irrigation (Grygier et al., 2002). Accompanying with geology, each temporary pool has its specific hydroregime, but all of which can be viewed to be stochastic. The favorable period for aquatic organisms is generally limited and fluctuated, reducing the importance of biotic factors (i.e. interspecific interactions) as in permanent pools (Collinson et al., 1995; Dahms, 1995; Jocqué et al., 2007), and resulting in special fauna composition.

The aquatic community in temporary pools should face the fluctuant hydroregime. In this scenario, residential invertebrates mainly adapt by two ways: to emigrate out when environment turns to be unfavorable, or to stay in anyhow (Jocqué et al., 2010). The former is not a typical resident, but is more like a disperser. Insects such as diving beetles, notonectids, and so on, adopt this kind of behavioral strategy to escape from environmental catastrophes. The latter group, however, can not migrate to other habitats by itself, and thus can be seen as a true resident. Crustaceans including branchiopods, ostracods, and copepods, stand for this group. Since they are all purely aquatic living, they evolve some structural or physiological mechanisms to survive through unfavorable periods.

Among these crustaceans, one old lineage of the subclass Branchiopoda which is called the large branchiopod because of its relatively large and easily visible body, dominates in temporary pools. Although most species are freshwater inhabiting, some lives in brackish water or even saline lakes, such as worldwide brine shrimps *Artemia* and Australia endemic *Paratemia* (Brendonck et al., 2008). Facing the unpredictable inundation and unstable hydroperiod, large branchiopods evolve several strategies to overcome the environmental difficulties. In general, they will hatch, grow up and become mature rapidly when the habitat turns to be suitable (Hildrew, 1985; Hamer and Appleton, 1991; Brendonck, 1996; Simovich and Hathaway, 1997; Weeks et al., 1997). Nevertheless, they still face the hardness during unfavorable periods. In addition to the life histories of free swimming stage, they produce eggs which are enveloped with several layers of membranes (i.e. the dormant stage) to bridge through the adverse period (Fryer, 1996; Hairston, 1996; Hairston and Cáceres, 1996; Brendonck and De Meester, 2003; Mura, 2004; Hulsmans et al., 2006).

One special character of dormant eggs is that they will not all hatch during a single hydroperiod, but will separate into several inundations. This kind of partial and gradual hatch has been proposed to be the bet hedging (Saiah and Perrin, 1990; Simovich and Hathaway, 1997; Brendonck et al., 1998; Menu et al., 2000; Schwartz and Jenkins, 2000; Mura, 2001; Brendonck and De Meester, 2003; Ripley et al., 2004; Maffei et al., 2005; Hulsmans et al., 2006; De Roeck et al., 2010) which acts as a risk spreading strategy to reduce the harm of abortive hatching, wherein the population will crash if all the eggs hatch but are not able to mature in time (Brendonck, 1996). Eggs which remain in dormancy and show delayed hatch will accumulate to form a highly generation overlapping egg bank in the sediment. Functionally, egg bank serves as a biotic reservoir, enabling population to escape temporally from unfavorable period (Gyllström and Hansson, 2004; Vandekerkhove et al., 2005). It can be said that egg bank is one of the most important reasons why large branchiopods can persist in temporary pools.

The structure of egg bank results from long term population dynamics spatially in a habitat and temporally throughout inundations (Brendonck and De Meester, 2003). Being the key for large branchiopods to persist in stochastic temporary pools, egg bank should be preserved well not only in the optimal place of a pool, but also with optimal hatching fraction across hydroperiods. In spatial aspect, pool edge is hypothesized to be the best place for laying dormant eggs, so that eggs will hatch when the water is more abundant (Hildrew, 1985; Thiéry, 1997) and thus the hydroperiod maintains longer. To achieve this purpose, large branchiopods should display active peripheral aggregation. Nevertheless, they may not be able to do so due to restricted immersed area. Additionally, even when adults lay around the edge, eggs may be dispersed afterwards, deviating from ideal edge distribution. On the other hand of temporal aspect, egg bank size represents the future of a population under frequent catastrophes. Facing the depletion from multiple ways such as senescence, mortality, and predation (Brendonck and De Meester, 2003), it needs to be recruited by hatching into free swimming individuals and reproducing successfully. However, each cohort could be abortive, failing to mature before the hydroperiod ends unpredictably. Hence, hatching should occur in the appropriate time with suitable amount not only within a hydroperiod but also across inundations (Brendonck and De Meester, 2003; Jocqué et al., 2010) for reducing the harm of abortive hatching and keeping the egg bank.

As a typical temporary pool, Siangtian Pond in northern Taiwan has some distinct characteristics, e.g. deeper water depths while accompanying with shorter hydroperiods. Locating in the interchanging zone between subtropical and temperate climate, yearly precipitation of Siangtian Pond can be more than 2,000 mm. However, it is the massive rainfalls happened within a few days (usually bringing by typhoons) that can form a functional hydroperiod. Because of its bowl shaped basin, rains would accumulate and the pool could be more than 4 m in depth and 80 m in diameter. At the same time, due to the porous sediment, water level reduces fast and stably at a rate of 32 cm per day (Huang et al., 2010). Consequently, the hydroperiod of Siangtian Pond generally lasts for two to three weeks only, except when additional rainfalls happen in time to extend the hydroperiod (Huang et al., 2010). Therefore, Siangtian Pond is characterized by relatively huge water body but short lived hydroperiod, generating a highly fluctuating environment for inhabiting large branchiopods.

Researches on Siangtian Pond and the large branchiopods started from Lin and Chou (1991) which identified the fairy shrimp *Branchinella kugenumaensis* (Ishikawa, 1895) and preliminarily described its life history, coexisting clam shrimps, and the habitat. Following studies focused on the free swimming stage, exploring how *B. kugenumaensis* adopted flexible growth patterns, i.e. determinate and indeterminate growth in short and long hydroperiods respectively, to optimize the fecundity throughout varied hydroperiods opportunistically (Huang et al., 2010), and evaluating its fitness variation across cohorts (Huang et al., 2011). From another point of view, distribution study demonstrated that the three sympatric species including *B.*

kugenumaensis, clam shrimps *Eulimnadia braueriana* Ishikawa, 1895 and *Lynceus biformis* (Ishikawa, 1895), displayed significant separation both horizontally and vertically across two hydroperiods in 2008, and was supposed to be a kind of niche differentiation in space (Wang et al., 2012). In horizon, each species dominated separately in certain places without an obvious pattern, and these places changed irregularly everyday. In vertical, *B. kugenumaensis* was distributed superficially, but *L. biformis* was prevailed throughout deeper zones. Further study on feeding structures revealed differentiated food resource exploitation among species, in which *B. kugenumaensis* was filter, *E. braueriana* was filtering feeder with ability to scrap, and *L. biformis* was a pure scraper (Liu and Chou, 2015). Differentiation in not only space but also food may reduce potential interspecific competition. Moreover, phototatic experiments on *E. braueriana* showed that it changed the phototaxis from positive to negative when developing into juveniles, suggesting a shift from pelagic to benthic living in Siangtian Pond (Liu et al., 2016). The dormant stage, however, does not receive attention yet. Distributions of dormant eggs, egg bank sizes, and hatching phenologies remain to be clarified. Even for free-living adults, long-term survey through more hydroperiods is recommended to reveal general spatial utilization patterns.

Therefore, the main content of my dissertation consists of four chapters to explore the potential strategies which large branchiopods adopt for living in Siangtian Pond. Referring to the spatial patterns, the first chapter (Chapter 2) showed distributional patchiness and peripheral aggregating tendency of *B. kugenumaensis*, *E. braueriana*, and *L. biformis*, and the second chapter (Chapter 3) focused on egg bank spatial structure, evaluating egg bank sizes and distributions of these three species. Taking information from above two chapters, I discussed the effects of free swimming adults on the spatial patterns of egg banks, proposing limitations and opportunities on achieving theoretically optimal egg distributions. As for the hatching regime, the third chapter (Chapter 4) demonstrated the hatching phenologies of *B. kugenumaensis* and *E. braueriana* on hatching time within a hydroperiod and hatching amount throughout hydroperiods. Finally, the last chapter (Chapter 5) tested if the observed hatching regimes of *B. kugenumaensis* and *E. braueriana* were idealized, with an optimal hatching fraction predicted by bet hedging strategy. I discussed the advantages of adopting this kind of hatching regime, and suggested different strategies between these two species. As a whole, my dissertation provides some of the most basic but important ecological information when approaching temporary pools and large branchiopods, and further extensions of meta-analysis on more populations across habitats with diverse hydroregimes can help to indicate general adaptation strategies to this special freshwater system.

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Chapter 2: Where do they go? Distribution pattern of sympatric large branchiopods in Siangtian Pond, a temporary pool in Taiwan

Abstract

Zooplanktons are well known for patchy distribution in both marine and freshwater system. Distributional patchiness may relate with mating and reproduction in aquatic environments, especially for large branchiopods in stochastic temporary pools. This study aimed at the spatial patterns of free-living adults of the large branchiopods *Branchinella kugenumaensis*, *Eulimnadia braueriana*, and *Lynceus biformis* in Siangtian Pond at northern Taiwan. From 2012 through 2014, systematic samplings with vertical townet along parallel transect lines were conducted in four hydroperiods. Individual number of each species was recorded for testing the patchiness in the whole pool, crowding among patches, edge aggregation, and the general hotspots in the basin. I found that the distribution in Siangtian Pond was patchy for all the three species, with more crowding in patches of *B. kugenumaensis* and *L. biformis*, probably benefitting their bisexual reproduction. Referring to edge aggregation, only *B. kugenumaensis* and *E. braueriana* displayed significant peripheral tendency, and this trend in the former one was consistent throughout hydroperiods, but varied in the latter species. Generally, hotspots of *B. kugenumaensis* and *L. biformis* located in the three deepest places of Siangtian Pond, and I suggest that this phenomenon was shaped by their late maturation time. In contrast, *E. braueriana* hotspots scattered widely throughout the basin and inclined towards the longitudinal path, which may relate with special preference to the less vegetated substrates. Comparing with egg bank structure, the inconsistency of spatial pattern between adults and eggs imply that in addition to peripheral aggregation of adults, redispersal of eggs could also lead to an optimal edge distribution of their eggs.

Keywords: *Branchinella kugenumaensis*, edge distribution, *Eulimnadia braueriana*, hotspot, *Lynceus biformis*, patchiness

Introduction

Patchy distribution is a common and well known phenomenon in both marine and freshwater zooplanktons (George, 1981; Omori and Hamner, 1982; Pinel-Alloul, 1995). These patches can range from millimeters to kilometers (Pinel-Alloul, 1995; Folt and Burns, 1999) and persist from hours to years (Omori and Hamner, 1982). Some could be less organized aggregations, but others could be highly organized and composed of a single species, sex, or even orienting to the same direction (Omori and Hamner, 1982). Potential mechanisms for zooplankton patchiness are quite diverse, including abiotic and biotic factors or their interactions. Generally, while large-scale and long-persistent patches are mainly affected by abiotic factors, biotic factors such as swarming behaviors can be more influential in smaller scales (Pinel-Alloul, 1995). In fact, functions of zooplankton patch are believed to be adaptive for the purposes of migration, foraging, anti-predation, mating, and reproduction (Ratzlaff, 1974; Omori and Hamner, 1982; Byron et al., 1983; Paffenhöfer et al., 1987; Davis et al., 1992).

In branchiopods, cladocerans (Branchiopoda, Cladocera) are small crustaceans and represent a group of zooplanktons in freshwater. Although distribution and patchiness of cladocerans have long been studied, these kinds of information are few for their relatives in temporary pools, the large branchiopods such as fairy shrimps (Anostraca), tadpole shrimps (Notostraca), and clam shrimps (Laevicaudata, Spinicaudata, Cyclestherida). Hildrew (1985) and Lindholm et al. (2012) simply mentioned that the fairy shrimp *Streptocephalus vitreus* (Brauer, 1877) and *Branchinecta paludosa* (Müller, 1788) swarmed, respectively. Other studies demonstrated diverse distribution patterns of large branchiopods in local habitats. They distributed either peripherally (Dexter and Ferguson, 1943; Knoll, 1995; Wang et al., 2012) or centrally (Khalaf and Hall, 1975), or had specific tendency towards both pool edge and center, depending on species (Hamer and Appleton, 1991). Nevertheless, degree of patchiness has not been quantified horizontally and vertically.

Between horizontal and vertical aspects, the latter is limited by shallow depth in temporary pools to demonstrate stable stratification and (diel) vertical migration, while the horizontal distribution could reflect specific adaptation to the characteristic stochasticity of temporary pools. Since the hydroperiod is unpredictable, eggs of large branchiopods would be better to hatch when the hydroperiod is expected to be long enough until they mature. To achieve this, pool edge is hypothesized to be the best place for laying eggs. This “strategy” can make sure that these peripheral laid eggs would hatch only if the water is abundant enough to cover the marginal regions (Hildrew, 1985; Thiéry, 1997). Therefore, it is reasonable to predict that large branchiopods would display pool edge distribution.

Siangtian Pond has unusual water depth fluctuation in temporary pools, ranging through several meters (Huang et al., 2010). Wang et al. (2012) studied the spatial distributions of sympatric large branchiopods including *Branchinella kugenumaensis* (Ishikawa, 1895), *Eulimnadia braueriana* Ishikawa, 1895, and *Lynceus biformis* (Ishikawa, 1895) in this temporary pool from both horizontal and vertical aspect. However, they failed to demonstrate clear horizontal patterns (Wang et al., 2012), probably due to the relatively few surveys across only two hydroperiods, with one especially short hydroperiod and abortive cohorts of *B. kugenumaensis* and *E. braueriana*. In addition, it is hard to suggest if the edge distribution of *B. kugenumaensis* (Wang et al., 2012) is a stable tendency or just happened during the survey periods. Long-term monitor throughout more hydroperiods can help to reveal if specific horizontal distribution exists and confirm if the tendency of peripheral distribution is stable.

This study focused on the horizontal distribution of *B. kugenumaensis*, *E. braueriana*, and *L. biformis* in Siangtian Pond, Taiwan. I aimed to clarify the distributional patchiness, edge distribution tendency, and daily changes in horizontal patterns of these three species. Hotspots in the basin were also explored throughout hydroperiods. Comparison between the egg banks and free swimming adults revealed limitation of available immersed areas and potential benefit of egg redispersal for large branchiopods to achieve optimal egg distribution toward the edge.

Materials and Methods

Study site

Siangtian Pond locates in the most northwest site of Yangmingshan National Park. The water source comes from precipitation only. Large-scale precipitations happening within short duration, usually the extremely heavy rains (> 200 mm/day, following the definition of Central Weather Bureau) brought by typhoons, can inundate the basin with several meters of water. Although the maximum water depth varies across hydroperiods, water level reduces at a stable rate of 32 cm per day (Huang et al., 2010). Figure 1A shows the characteristic landscapes of Siangtian Pond, including the 4 m inundation area, a hiking path, and the three deepest places (the “ecological centers”) which still hold water in the last day of a given hydroperiod. Vegetation is widespread and dense throughout the basin except near the path (Wang et al., 2014). Inundation area changes significantly through the depth, even separating into two regions when water depth is less than 2 m (Fig. 1B). Large branchiopods *B. kugenumaensis*, *E. braueriana*, and *L. biformis* live in, sometimes co-occurring (Wang et al., 2012).

Field sampling

Field samplings were conducted whenever Siangtian Pond was inundated. Six hydroperiods (2012P1, 2012P2, 2013P1, 2013P2, 2013P3, and 2014P1) happened and were surveyed from August 2012 to August 2014. However, two of them (2012P2 and 2013P2) were abortive hatchings, and only data of four hydroperiods (2012P1, 2013P1, 2013P3, and 2014P1) were included in this study. Determination of when the population matured followed Wang et al. (2014), defining the day which more than 50 % females (*B. kugenumaensis* and *L. biformis*) or hermaphrodites (*E. braueriana*) had well enveloped eggs in the brood pouch (*B. kugenumaensis*) or dorsal side (*E. braueriana* and *L. biformis*) as maturation time (DAII: days after initial inundation).

During daily survey, I applied parallel transect lines to cover entire inundation area, with distance of 12-15 m between lines. Sampling was taken every 12-15 m along each transect line. It should be noticed that the transect line was not always straight, but would be modified accordingly in the pool edge (Fig. 2A). Individuals were sampled vertically by a townet (diameter: 14.5 cm, mesh size: 0.1 mm) in each sampling point. A heavy plumb was tight at one side of the townet to let it sink vertically to the bottom of Siangtian Pond. The open aspect of townet faced horizontally when sinking (Fig. 2B), preventing any individual from being caught. After the townet laid on the bottom, I waited for 30 seconds to reduce potential disturbance, drew the townet vertically to the surface, and counted the individual number ($n_{i,j}$; i: the i^{th} DAII, j: the j^{th} sampling point) of each species. Alive animals were then released back to the water. During the last few days when the maximum water depth was less than 1 m, a handy plankton net (perimeter: 14.5 × 12 cm, mesh size: 0.1 mm) was used alternatively. Similarly, I put the plankton net vertically to the bottom, keeping the open aspect horizontally during the process, waited for 30 seconds, sampled vertically to the surface, and counted the individual number ($n_{i,j}$). GPS coordinates (Garmin, Dakota 20) of each sampling point were recorded at the same time

Distributional patchiness in entire pool

First, density ($d_{i,j}$, individual number/m^2) of each species on each sampling point was calculated by

$$d_{i.j} = \frac{n_{i,j}}{A} \times 10000 \quad (1),$$

in which A is the area of the townet or the plankton net (cm^2). Timing 10000 transfers the unit from cm^2 to m^2. Since I aimed at the horizontal distribution and the hotspots of adults, density in area rather than in volume was used.

I followed Lloyd's index (1967), ratio of mean crowding to mean density ($\frac{\overset{*}{d_i}}{d_i}$) to indicate patchiness. This index had been proved to be density independent (George, 1974). Mean crowding ($\overset{*}{d_i}$) was calculated by

$$\overset{*}{d_i} = \overline{d_i} + \left(\frac{s_i^2}{\overline{d_i}} - 1\right) \quad (2),$$

wherein $\overline{d_i}$ and s_i^2 represent the mean density and variance of density of a given species in the i^{th} DAII, respectively. The distribution is patchy when the index value ($\frac{\overset{*}{d_i}}{\overline{d_i}}$) is significantly higher than 1. To test the significance of Lloyd's index, I applied one-sample Kolmogorov-Smirnov test (comparing with an assumed normal distribution, mean = 1 and standard deviation = 0.5) because the sample size was too small to perform reliable Chi-square test. The index value was further compared between species by Kruskal-Wallis test due to either not normally distributed or not equal variances.

Crowding of patches

To know how each species of large branchiopods aggregated in those patches with individuals, I created a "cumulative concentration curve" to demonstrate the crowding level. For *k* patches with individuals, I hierarchically ranked them from the highest to the lowest individual number of in a given day, transferred into the percentage of individuals caught at each patch, and accumulated sequentially as P_k (%). The P_k was use as Y axis and plotted against the X axis, P (%), which was 100 % dividing by k and was then accumulated sequentially. It should be noticed that k varied through the day. Afterwards, regression between P and P_k was examined. If it is logarithmical, individuals are more concentrated in a few patches. Contrarily, if it is linear function, distribution is relatively even among patches. The correlation between P and P_k was explored by general linear model (GLM).

Edge aggregation

Edge was defined as the range within 30 cm away from the boundary between wet and dry area. I calculated percentage of individuals in the edge in a given day, and compared with the expected random percentage in the edge by two-sample Kolmogorov-Smirnov test due to small sample sizes. Because inundation area changed through the day, the expected percentage in the edge would vary accordingly. In addition, differences of edge percentages between species were tested by Kruskal-Wallis test due to either not normally distributed values or not equal variances.

Horizontal distribution patterns and hotspots

To clarify the horizontal distribution through the day and the overall pattern, $d_{i,j}$ of a given species was plotted against the GPS coordinates across the day and throughout the four hydroperiods, respectively. 2-D scatterplot was plotted with $d_{i,j}$ through all of the hydroperiods to reveal the hotspots of each species. Due to highly diverse $d_{i,j}$, trying to fit all of the $d_{i,j}$ will result in over rugged contours and blur the real pattern. Therefore, distance-weighted least-squares (DWLS; McLain, 1974), which fits a least-square regression line for a group of points by decreasing the effect of points through the distance to the line, was applied. DWLS allows common values to be more important than uncommon ones, so it is more sensitive to non-notable data, showing the underground distribution pattern.

All the analyses were performed by SYSTAT 12.

Results

Table 1 summarizes the inundation episode, hydroperiod, and maturation time of each species. 2012P1, 2013P1, 2013P3, and 2014P1 lasted for 17, 14, 24, and 26 DAII (days after initial inundation), respectively. *Eulimnadia braueriana* was the earliest maturing species, carrying the first clutch of eggs at 9 DAII. *Branchinella kugenumaensis* was the second one, maturing at 10-11 DAII. *Lynceus biformis* was the last at 13-15 DAII, and did not reach maturity in 2013P1.

Lloyd's patchiness index (ratio of mean crowding to mean ratio index, $\frac{\overset{*}{d}_i}{d_i}$) for *B. kugenumaensis*, *E. braueriana*, and *L. biformis* was 4.1 ± 1.8, 5.7 ± 4.1, and 2.7 ± 1.2, respectively, and were all significantly higher than 1 (one-sample Kolmogorov-Smirnov, maximum difference = 0.92, 0.96, and 0.8, $p < 0.001$ for *B. kugenumaensis*, *E. braueriana*, and *L. biformis* respectively), indicating patchy distribution for these three species. Among them, *B. kugenumaensis* and *E. braueriana* showed a significantly higher index value than *L. biformis* (Kruskal-Wallis, $\chi^2 = 8.7$ and 8.2, $p < 0.01$ for *B. kugenumaensis*/*L. biformis* and *E. braueriana*/*L. biformis*, respectively), while there had no significant difference between *B. kugenumaensis* and *E. braueriana* (Kruskal-Wallis, $\chi^2 = 2.9$, $p = 0.09$).

Cumulative concentration curves are showed in Figure 3. The regression was logarithmic in both *B. kugenumaensis* and *L. biformis* (general linear model, $r^2 = 0.88$ and 0.93, $p < 0.001$ for *B. kugenumaensis* and *L. biformis*, respectively; Fig. 2A, 2C), but was linear in *E. braueriana* (GLM, $r^2 = 0.87$, $p < 0.001$; Fig. 2B). These results mean that *B. kugenumaensis* and *L. biformis* were more clumped within fewer patches, with about 70 % of individuals distributing in 30 % of patches. *Eulimnadia braueriana*, on the contrary, was less crowded.

Observed and expected percentages of individuals locating in the pool edge throughout the four hydroperiods are shown in Table 2. Averaged percentage in the edge was 82.2 ± 13.1 %, 70.1 ± 22.8 %, and 74.2 ± 17.2 % for *B. kugenumaensis*, *E. braueriana*, and *L. biformis*, respectively. It was significantly different from random distribution in *B. kugenumaensis* and *E. braueriana* (two-sample Kolmogorov-Smirnov, maximum difference = 0.71 and 0.34, $p < 0.001$ and 0.05 for *B. kugenumaensis* and *E. braueriana*, respectively), but was not in *L. biformis* (two-sample Kolmogorov-Smirnov, maximum difference = 0.42, $p = 0.06$). For the comparisons between species, *B. kugenumaensis* had a generally higher edge percentage than *E. braueriana* (Kruskal-Wallis, $\chi^2 = 4.3$, $p < 0.05$) and *L. biformis* (Kruskal-Wallis, $\chi^2 = 4.4$, $p < 0.05$), while no significant difference happened between *E. braueriana* and *L. biformis* (Kruskal-Wallis, $\chi^2 = 0.3$, $p = 0.57$). In other words, more *B. kugenumaensis* and *E.*

braueriana adults distributed peripherally, and *B. kugenumaensis* showed the highest proportion in the edge among these three species.

Density ($d_{i,j}$) distributions in horizon are shown across the day for each species from Figure 4 through Figure 6. *Branchinella kugenumaensis* displayed a consistent edge distribution, except during the last few days of a given hydroperiod and 2013P1 which persisted shorter and had a smaller inundation area (Fig. 4; Table 2). However, it did not distribute in specific peripheral region and changed throughout. On the other hand, both *E. braueriana* and *L. biformis* did not show the similar distribution as *B. kugenumaensis*. Regions with higher $d_{i,j}$ changed between the center and periphery or happened across wider regions irregularly throughout, without an obvious pattern (Fig. 5; Fig. 6). Furthermore, *E. braueriana* was very patchy during the last few days of a given hydroperiod (Fig. 5). It should be noticed that the $d_{i,j}$ of *E. braueriana* was generally less than tenfold comparing with *B. kugenumaensis* and *L. biformis*.

Horizontal distributions throughout the four hydroperiods and the hotspots for each species are shown from Figure 7 through Figure 9. The distributional hotspots of *B. kugenumaensis* were mainly concentrated in the three deepest places and expanded a little bit towards the northeast margin (Fig. 7). In contrast, *E. braueriana* hotspots were more widespread across the whole basin of Siangtian Pond, especially near the eastern longitudinal path. It distributed through most peripheral regions, except the southwest part (Fig. 8). Similar to *B. kugenumaensis*, hotspots of *L. biformis* chiefly located at the three deepest places, and part of the eastern marginal regions (Fig. 9).

Table 3 summarizes the major results. From the view of entire pool, all the three species of large branchiopods in Siangtian Pond showed patchy distribution. In the aspect of crowding, *B. kugenumaensis* and *L. biformis* were more clumped in patches with individuals, while *E. braueriana* distributed evenly throughout the patches. Regarding to edge aggregation, only *B. kugenumaensis* and *E. braueriana* displayed significant tendency. This edge aggregating tendency was daily consistent in *B. kugenumaensis*, but varied throughout in *E. braueriana*. Overall, the distributional hotspots of *B. kugenumaensis* and *L. biformis* were focused in the three deepest places, while it was more dispersed across the whole basin, with some inclination towards the eastern longitudinal path in *E. braueriana*.

Discussion

Comparing to the larger freshwater system such as lakes, temporary pools are relatively small-scale habitats with significant stochastic hydroregimes and fluctuating environments for inhabiting organisms. The heterogeneity of temporary pools can influence the biota in fine scale, but has not been studied in detail yet. Living in temporary pools, large branchiopods should adapt by corresponding distribution in terms of patchiness in the whole pool, crowding among patches, edge aggregation, and specific hotspots. Clarifying their distribution pattern through hydroperiods can help to reveal the possible benefits when adults utilize the available area, as well as the restriction from both external environment and internal development. In addition, comparing horizontal distribution between free-living individual and egg bank can clarify if the habitat utilization of free-living stage determines the structure of dormant stage, or other factors can also be influential.

Although Siangtian Pond has some significant landscapes such as the regions with dense common rush, maiden grass, and artificial path (Wang et al., 2014), I surveyed across the whole inundation area rather than specifically on these landscapes. The uneven sampling efforts and diverse densities may blur the real hotspots when I took all the data throughout hydroperiods together, but results can indicate the general pattern of each species in the basin. On the other hand, I only divided the microhabitats into edge and non-edge without detailing other environmental characters. Living in the unstable hydroperiod, large branchiopods should reproduce as early as possible before the habitat dries out (Hildrew, 1985; Brendonck et al., 2000). In this situation, to lay eggs in the pool periphery could be the best strategy for ensuring that the egg would hatch when water is abundant to reach the margin so that the hydroperiod is longer (Hildrew, 1985; Thiéry, 1997). Thus, the roughly two categories on Siangtian Pond are enough for understanding if they distribute optimally towards the edge.

In my study, all the three species of large branchiopods showed patchy distributions. Since Siangtian Pond is characterized by the deeper water depth in temporary pools ranging through several meters and rapid water reduction rate of 32 cm per day (Huang et al., 2010), available microhabitats for large branchiopods will change accordingly and quickly. Uneven topography and vegetations complicate the microhabitats furthermore. In addition to the environment, potential biotic interactions such as the spatial differentiation among species (Wang et al., 2012) can also act on distribution, resulting in patchiness formation. However, clam shrimp *L. biformis* was the least patchy comparing to other two species. Laevicaudatans not only frequent the bottom but also swim or grasp aquatic plants (Fryer and Boxshall, 2009), mainly living in vegetation (Dumont and Negrea, 2002). The widespread vegetation through the basin of Siangtian Pond (Wang et al., 2014) may provide *L. biformis* with preferable microhabitats and lower the distributional patchiness.

Referring to the crowding among patches, however, *B. kugenumaensis* and *L. biformis* were more clumped than *E. braueriana*. In zooplanktons, adaptive advantage of clumping individuals includes many different aspects such as foraging, dispersal, and anti-predation (Omori and Hamner, 1982), while reproduction and mating are usually the most important reason (Ratzlaff, 1974; Byron et al., 1983; Davis et al., 1992). Our results of large branchiopods could be realized from their reproductive system. Bisexually reproductive *B. kugenumaensis* and *L. biformis* would benefit from clumping, increasing the efficiency for finding mates and mating in the stochastic hydroperiods. Contrarily, *E. braueriana* was less concentrated through patches. Considering that the population majorly composed of self-fertilizing hermaphrodites, advantage for aggregating may be more reduced in *E. braueriana*, or even worse because clumping leads to competition for food.

Viewing pool edge as the ideal place for large branchiopods to lay their eggs, my results demonstrated that *B. kugenumaensis* and *E. braueriana* distributed peripherally, but no significant difference from random distribution between edge and non-edge in *L. biformis*. Throughout the day, only *B. kugenumaensis* showed a consistent peripheral distribution, although regions of high abundance changed daily. Among the three species, *B. kugenumaensis* had the highest proportion of individuals in the edge. I confirmed Wang et al. (2012)’s observation which showed peripheral *B. kugenumaensis*. It indicates that *B. kugenumaensis* tend to distribute peripherally, while this is not the same meaning as the “peripheral species” in Hamer and Appleton (1991), stably living in the edge of pool basin with specialized life histories. Because the inundation area of Siangtian Pond changes rapidly through the day, edge regions vary accordingly. *Branchinella kugenumaensis* consistently clump in the edge, even though the peripheral environment modifies from day to day. Potential mechanisms for edge distribution include that wind blew individuals to the leeward (Dexter and Ferguson, 1943), or that branchiopods favored specific vegetation and depths (Hamer and Appleton, 1991). I speculate that the consistence in *B. kugenumaensis* results from active behavior. Since the microhabitat changes every other day, preference to given plants or depths may not lead to this kind of stability in distribution. Moreover, not towards specific peripheral regions diminishes the impact of wind blowing, which is usually directional when the northeast monsoon predominates after autumn in Siangtian Pond. Special mating behavior of freshwater fairy shrimps may be responsible for edge aggregation. I hypothesize that the hilltopping behavior (Scott, 1968), originally described in insects such as butterflies, exist in *B. kugenumaensis* and cause the peripheral distribution.

In the scramble-competition polygyny mating system (Alcock, 1980) of nonartemian anostracans, males will eagerly search for females by approaching any object nearby without discrimination (Wiman, 1981; Belk, 1991). Although the signals using for mating are imprecise (Wiman, 1981; Belk, 1991), vision is crucial for guiding mating behavior and leading to mating success (Belk, 1991). Superficial distribution of *B. kugenumaensis* (Wang et al., 2012) may relate to the need of light for finding females visually. The edge regions could serve as the hilltop, providing a remarkable highland for patrolling males to aggregate. Moreover, receptive females will make themselves more available to mates (Belk, 1991), possibly by staying near the surface (Pearse, 1913). As a whole, nature of searching and waiting for mates in males and females respectively, would gradually accumulate individuals and hold the aggregations stably in edge of inundation regions. Nevertheless, these peripheral aggregations need not to be fixed. This may explain why the places of these patches were irregular, similar to Wang et al. (2012).

Eulimnadia braueriana also distributed peripherally, but without consistency. Since the majority of population composes of hermaphrodites, the effect of mating behavior on distribution could be less possible. Phototactic behavior changed from positive to negative through development (Liu et al., in press) may lead pelagic nauplii to the edge and become benthic living there. However, negatively phototactic adults may redistribute during the night, while its poor swimming ability by second antennae only (Dumont and Negrea, 2002; Fryer and Boxshall, 2009) may retard the dispersal back to the edge, and so was the inconsistent pattern of *E. braueriana*.

On the other hand, *L. biformis* did not show specific distribution towards edge or non-edge. Similar to what Wang et al. (2012) demonstrated, it distributed widely throughout the inundated basin without an obvious pattern. Mating behavior of *L. biformis* is different from *B. kugenumaensis*. Although the effect of physical contact and chemical cues is not as clear as *Eulimnadia* spp. (*Eulimnadia texana* (Parker, 1978); Weeks and Benvenuto, 2008), visual signals have not been suggested to function during mating process (Sigvardt and Olesen, 2014). It may not be able to select the remarkable edge as the hilltop for actively aggregating by vision. Places for *L. biformis* to clump and mate could be relatively random in the widespread vegetation. Further studies on mating, feeding, and locomotion can help to clarify their dispersal and distribution mechanisms in Siangtian Pond.

In addition to daily distribution, distributional patterns and hotspots were different among species. *Branchinella kugenumaensis* and *L. biformis* had similar distributional hotspots in the three deepest places of Siangtian Pond basin, while hotspots of *E. braueriana* distributed widely towards most edge regions and were the highest near the eastern longitudinal path. Although *B. kugenumaensis* showed periphery distribution, late maturation restricted the available range it could use. Limited water body during the last period of a given hydroperiod would concentrate individuals in these deepest places at high densities, resulting in the contrary between daily and overall distribution pattern. Likewise, the last maturing *L. biformis* had even more limited utilization area than *B. kugenumaensis*, and the hotspots also located at the deepest places. *Eulimnadia braueriana* was the earliest species to become mature, so it could use broader area. Accompanying with significant peripheral distribution, its hotspots reached most edge regions of Siangtian Pond. However, inclination towards the artificial path still implies specific microhabitat preference. Benthic living, filter feeding with certain ability for scraping (Liu et al., in press) may let *E. braueriana* to prefer the less vegetated and naked substrates. Moreover, the path may provide suitable places for reproduction if it has the same behavior as *E. texana*, burrowing and laying eggs underground (Knoll, 1995). As a whole, *E. braueriana* benefits from early maturation and is able to distribute throughout the basin, while hotspots of *B. kugenumaensis* and *L. biformis* locate centrally in the three deepest places, regardless of their daily distributions.

Living in Siangtian Pond, large branchiopods face diverse sources of limitation. Unpredictable amount of water at the beginning of a given hydroperiod determines the inundation area at first. High water reduction rate decreases the area afterwards. Population maturation time restrict potential range furthermore. While they experience the external and internal limitation from environment and development respectively, specific mechanisms may provide opportunity to optimize the microhabitat utilization, distributing peripherally towards ideal regions for laying eggs (Hildrew, 1985; Thiéry, 1997). *Eulimnadia braueriana* achieves by early maturation and edge distribution. *Branchinella kugenumaensis*, on the other hand, reaches the same purpose by consistent edge distribution. Although it is late maturing and usually has smaller utilizing area, the significant edge aggregation can help *B. kugenumaensis* to lay eggs in the most optimal regions of a given hydroperiod under its developmental limitation. The advantage of peripheral distribution will be more evident if hydroperiod is extended. In occasionally prolonged hydroperiods such as 2004P3 and 2008P2 (Huang et al., 2010), *B. kugenumaensis* could expand the egg laying area towards more peripheral regions of Siangtian Pond, probably benefitting this poor hatching species (Wang et al., 2015) from putting eggs in the edge. Generally short-lived *E. braueriana* (Wang et al., 2012) and not edge aggregating *L. biformis* may have more limited ability to lay eggs peripherally in prolonged hydroperiods.

Comparing the distributional hotspots of this study with horizontal egg distribution (Wang et al., 2014), it reveals different mechanisms for each species to construct the egg bank spatial structure. In *B. kugenumaensis*, hotspots of both adults and eggs were focused in the three deepest places of Siangtian Pond, supporting the suggestion that the utilization pattern of adults basically determines the egg bank structure (Wang et al., 2014). However, the distributional pattern of the other two species did not fit between free-living adults and dormant eggs. Although hotspots similarly located around the less vegetated path, they inclined towards eastern and western part for adults and eggs respectively in *E. braueriana*. I speculate that artificial influence of hiking along the path would disturb the egg bank unevenly and result in the inconsistency. Moreover, the latest maturing *L. biformis* should have the highest restriction on available area within the three deepest places. Nevertheless, its eggs were found to be widely distributed towards the more peripheral regions. This contradiction indicates further dispersal after eggs are laid. Factors such as wind (Thiéry, 1997; Mura, 2004; Pinceel et al., 2015) and egg flotation (Thiéry, 1997; Gyllström and Hansson, 2004; Pinceel et al., 2013) may also be influential. In general, not only the active microhabitat utilization of adults but also the passive redispersal of eggs can act on and complicate the interaction between free-living and dormant stage, offering various limitations and opportunities for large branchiopods to adapt to the habitats.

To conclude, all the three species of large branchiopods showed patchy distribution in Siangtian Pond, while *B. kugenumaensis* and *L. biformis* were more concentrated in those patches with individuals, possibly the clump for mating. Only *B. kugenumaensis* displayed consistent edge distribution throughout the day, indicating that active behaviors related with mating may participate in. For *E. braueriana* and *L. biformis*, mechanisms such as locomotion and feeding could also affect and resulted in the inconsistent distribution towards periphery or unconspicuous pattern towards specific regions. Overall, the distributional hotspots of *B. kugenumaensis* and *L. biformis* located in the three deepest, long-lasted places due to late maturation. Hotspots of the earliest maturing *E. braueriana* distributed across most regions of the basin and inclined towards part of the artificial path, implying preference to less vegetated substrates. To lay eggs in the optimal pool edge, large branchiopods face different sources of limitations and opportunities from inundation area, maturation time, and peripheral aggregating behavior. Further dispersal after the eggs are laid may also affect egg bank spatial structure in horizon. As a whole, interactions between free-living adults and dormant eggs are dynamic and can be complicated by multiple factors.

Table 1. Inundation episode, hydroperiod (DAII: days after initial inundation), and maturation time (DAII) for *E. braueriana* (Eb), *B. kugenumaensis* (Bk), and *L. biformis* (Lb). MNR: maturity not reached.

Inundation	**Hydroperiod**	**Maturation time**		
		Eb	**Bk**	**Lb**
2012P1	17	9	11	15
2013P1	14	9	11	MNR
2013P3	24	9	10	13
2014P1	26	9	10	15

Table 2. Observed and expected percentages of individuals located in the pool edge throughout the four hydroperiods. DAII: days after initial inundation; Eb: *E. braueriana*, Bk: *B. kugenumaensis*, Lb: *L. biformis*.

Hydroperiod-DAII	**Eb**	**Bk**	**Lb**	**Expectation (Random)**
2012P3-9	70.6			56
2012P3-10	37.7			46.4
2012P3-11	30.3	83.3		48.4
2012P3-12	34	92		50
2012P3-13	81.4	89.5		50
2012P3-14	80	86.2		54.2
2012P3-15	100	76.2	65.9	65
2012P3-16	69.2	79.4	62	75
2012P3-17	100	88.1	98.9	83.3
2013P1-9	88.2			71.4
2013P1-10	62.5			75
2013P1-13	50	40.5		63.6
2013P1-14	100	72.9		68

2013P3-10	54.5	93.3		44.4
2013P3-11	90	90.2		44.4
2013P3-15	43.5	90.2	31.6	40
2013P3-16	35.3	79.7	50.5	41.9
2013P3-17	62.1	81.3	51.2	40.9
2013P3-18	73.7	85.4	73.3	41.9
2013P3-19	52.4	82.8	78.5	43.6
2013P3-20	60	74.4	75.9	45.5
2013P3-21	90	87.3	67.9	48.3
2013P3-22	76.9	76.3	79	52
2013P3-23	100	87.8	80.8	71.4
2013P3-24	100	96.6	96.9	85.7
2014P1-9	77.3			48.7
2014P1-10	64.7	94.9		46.2
2014P1-11	69.2	84.2		47.4
2014P1-12	61.1	94.6		50
2014P1-15	37.9	84.5	86.1	65.5
2014P1-18	50	87.9	74.5	73.1

2014P1-20	100	81.4	75.3	72.7
2014P1-22	50	72	85.4	67.9
2014P1-23	100	42.6	76.9	73.9

Table 3. Brief summary of results. Bk: *B. kugenumaensis*, Eb: *E. braueriana*, Lb: *L. biformis*.

	Bk	**Eb**	**Lb**
Patchiness in the whole pool	High	High	Mid
Crowding of patches	High	Low	High
Edge aggregation	Yes (consistent)	Yes (varied)	No
Hotspot in general	Three deepest places	Widespread with some inclination	Three deepest places

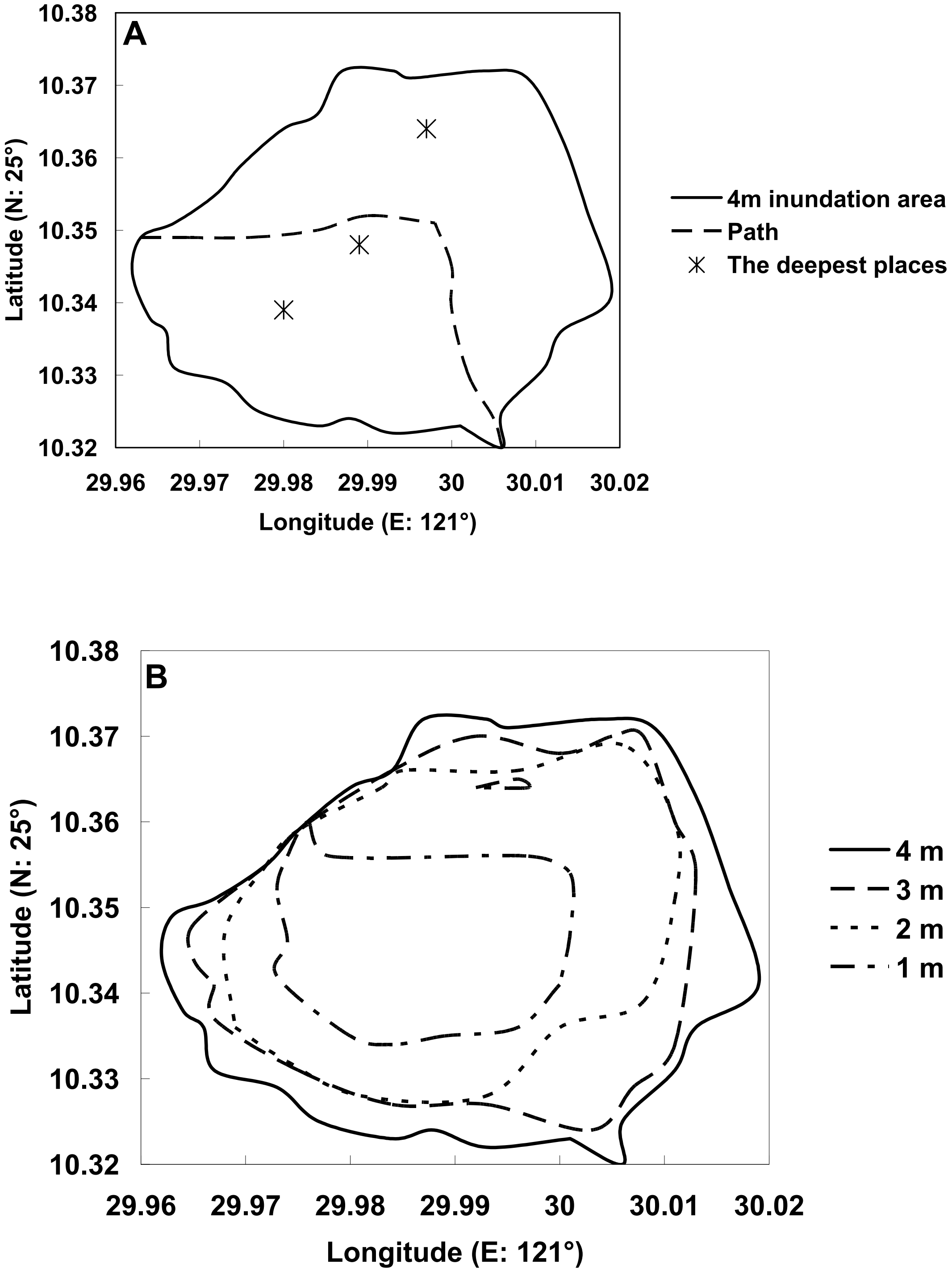


Figure 1. (A) Characteristic landscapes, and (B) changes of inundation area through water depth of Siangtian Pond.

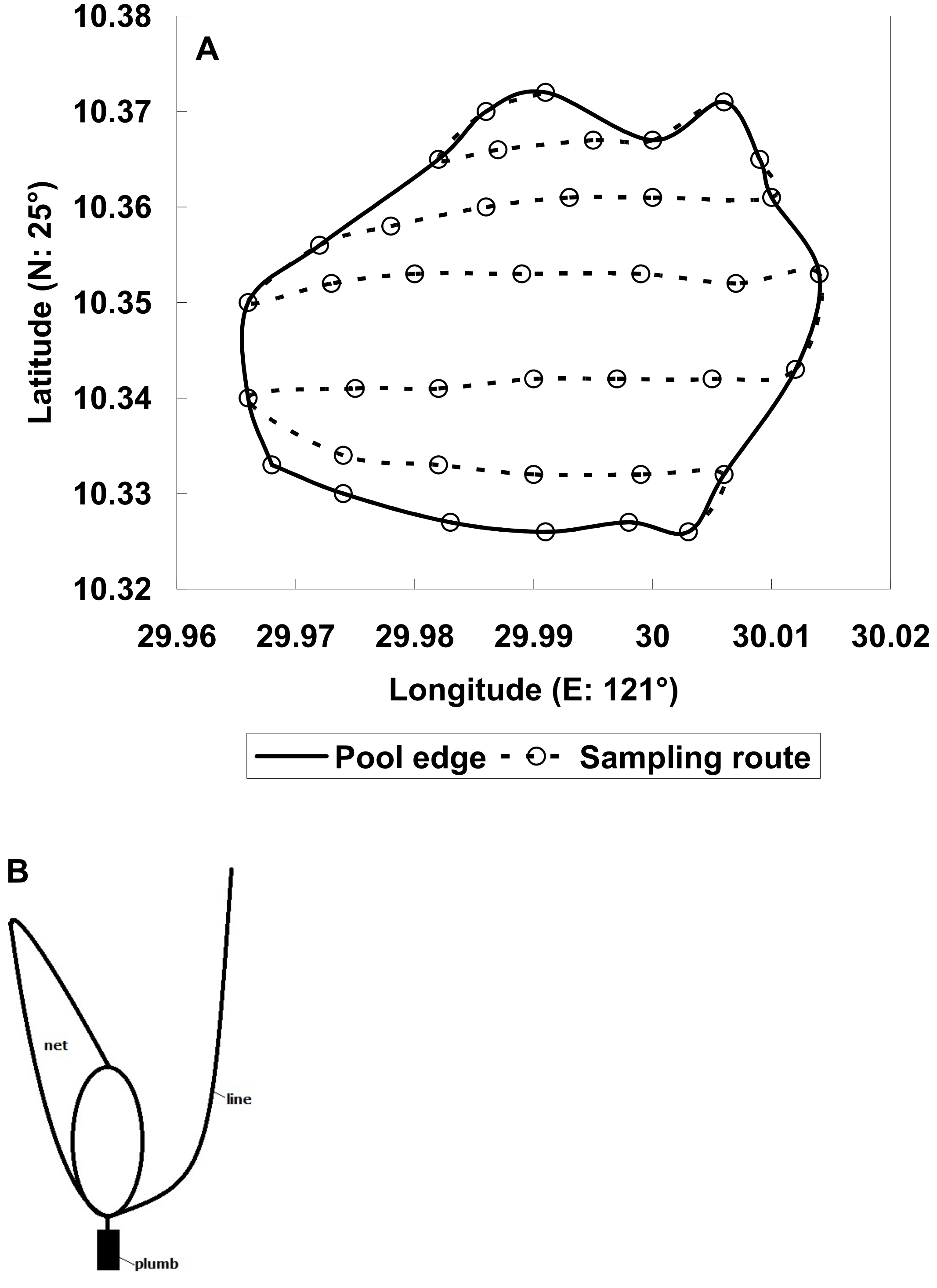
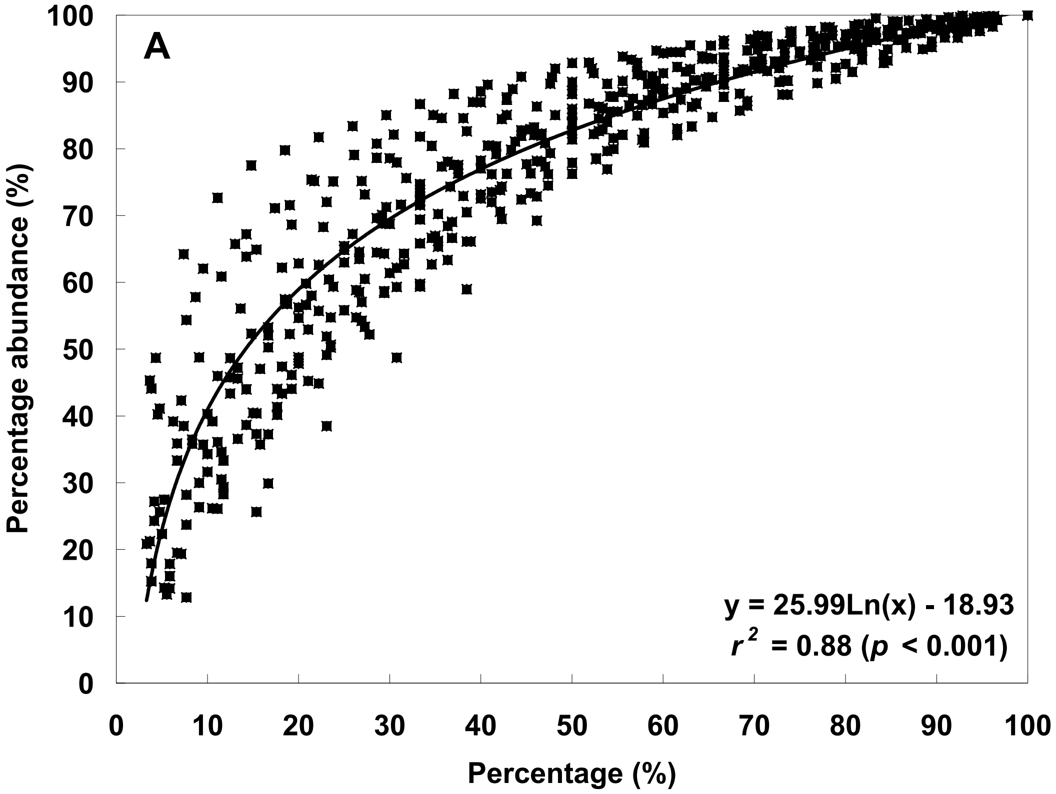
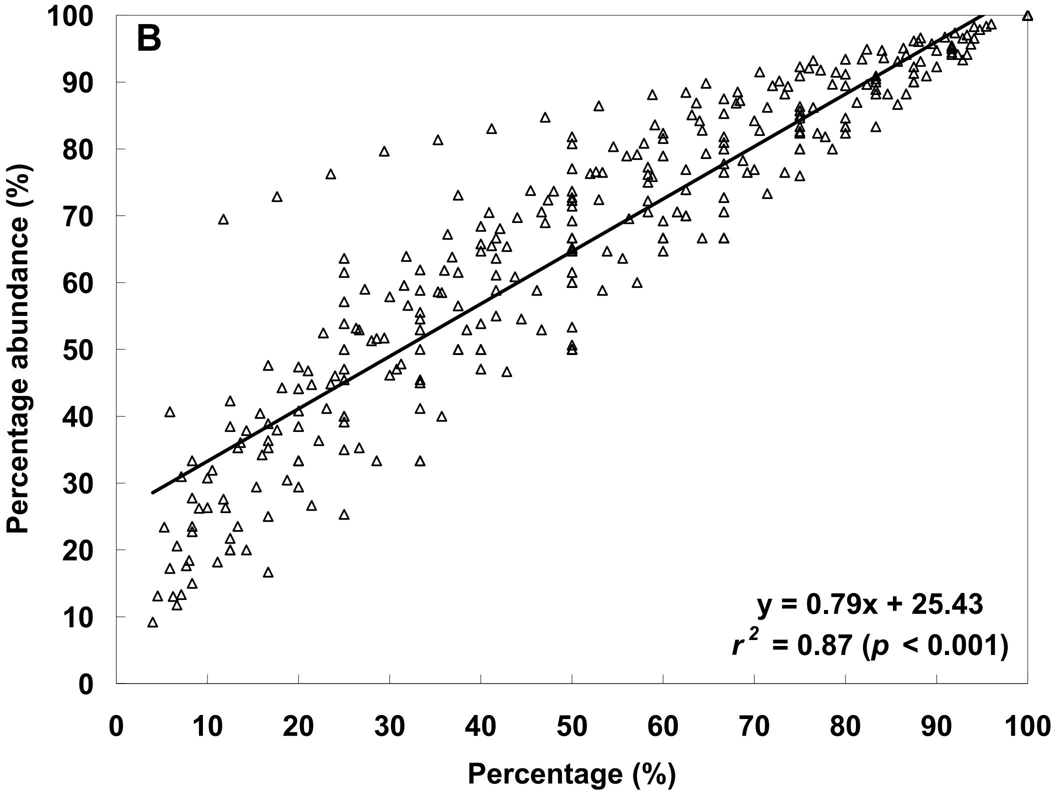


Figure 2. Diagram of (A) transect lines and sampling points, using the 9th DAII (days after initial inundation) of the hydroperiod 2014P1 as an example, and of (B) sinking townet.


A
Percentage abundance (%)
0
10
20
30
40
50
60
70
80
90
100
Percentage (%)
0
10
20
30
40
50
60
70
80
90
100
y = 25.99Ln(x) - 18.93
r² = 0.88 (p < 0.001)


B
Percentage abundance (%)
0
10
20
30
40
50
60
70
80
90
100
Percentage (%)
0
10
20
30
40
50
60
70
80
90
100
y = 0.79x + 25.43
r² = 0.87 (p < 0.001)

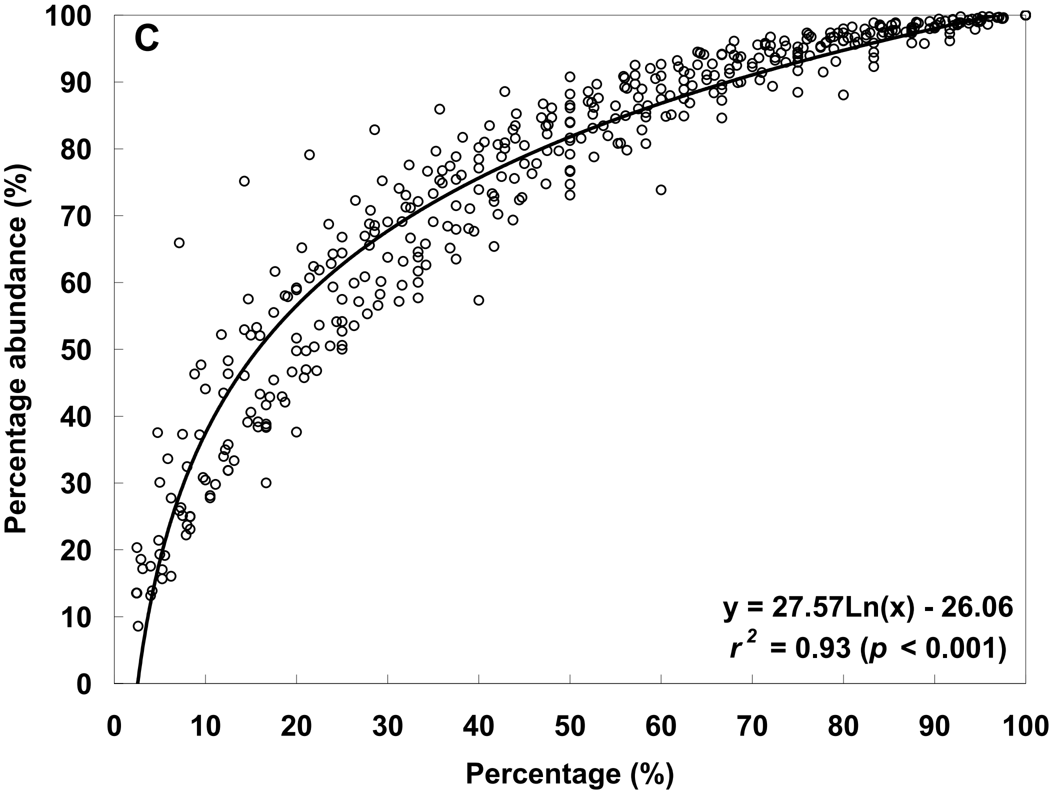
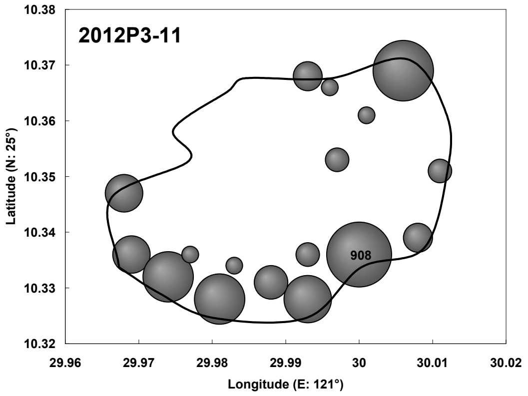
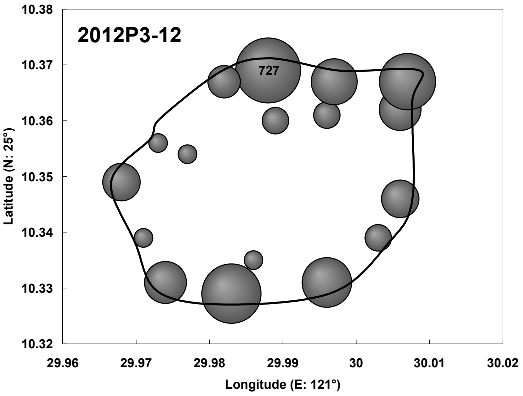
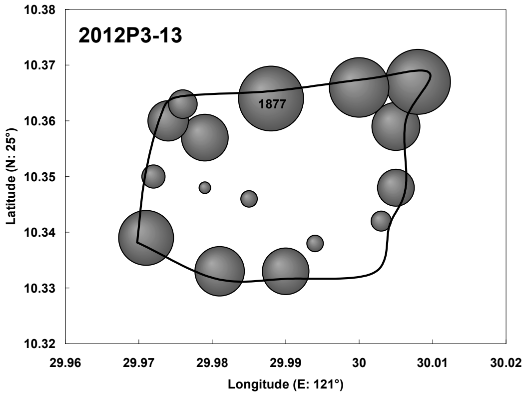
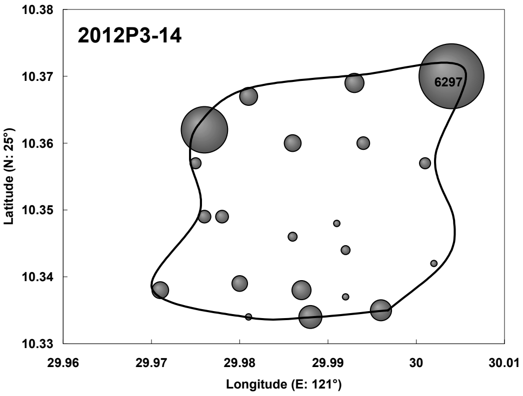


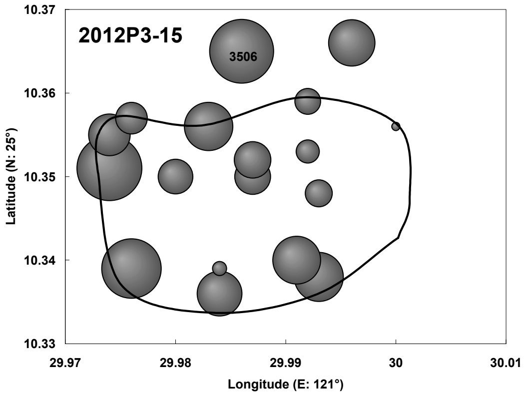
Figure 3. Cumulative concentration curve for (A) *B. kugenumaensis*, (B) *E. braueriana*, and (C) *L. biformis*.


2012P3-11
908
Latitude (N: 25°)
10.38
10.37
10.36
10.35
10.34
10.33
10.32
29.96
29.97
29.98
29.99
30
30.01
30.02
Longitude (E: 121°)


2012P3-12
727
Latitude (N: 25°)
10.38
10.37
10.36
10.35
10.34
10.33
10.32
29.96
29.97
29.98
29.99
30
30.01
30.02
Longitude (E: 121°)


2012P3-13
1877
Latitude (N: 25°)
10.38
10.37
10.36
10.35
10.34
10.33
10.32
29.96
29.97
29.98
29.99
30
30.01
30.02
Longitude (E: 121°)


2012P3-14
6297
Latitude (N: 25°)
10.38
10.37
10.36
10.35
10.34
10.33
29.96
29.97
29.98
29.99
30
30.01
Longitude (E: 121°)


2012P3-15
3506
Latitude (N: 25°)
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10.35
10.34
10.33
29.97
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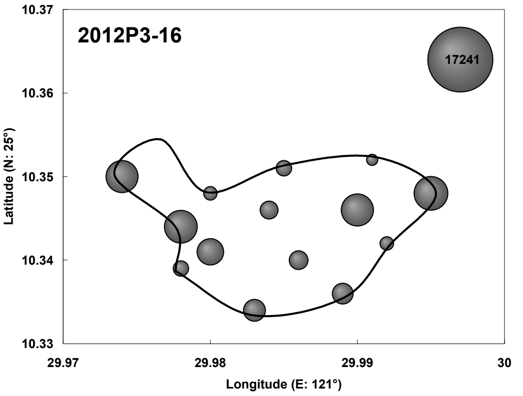
29.99
30
30.01
Longitude (E: 121°)


2012P3-16
17241
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10.36
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10.34
10.33
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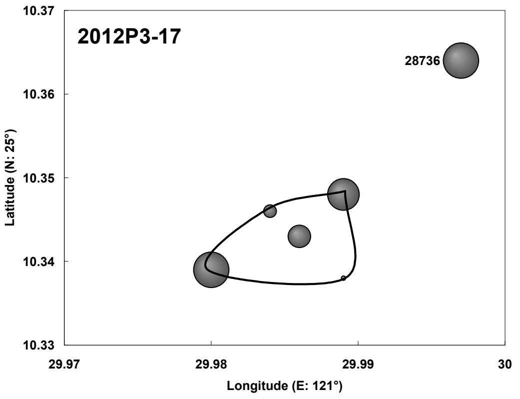
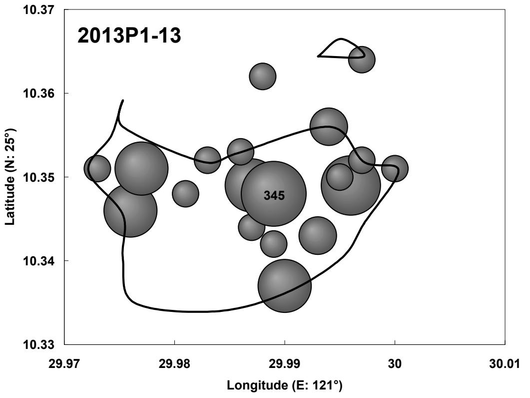
29.98
29.99
30
Longitude (E: 121°)


2012P3-17
28736
Latitude (N: 25°)
10.37
10.36
10.35
10.34
10.33
29.97
29.98
29.99
30
Longitude (E: 121°)


2013P1-13
345
Latitude (N: 25°)
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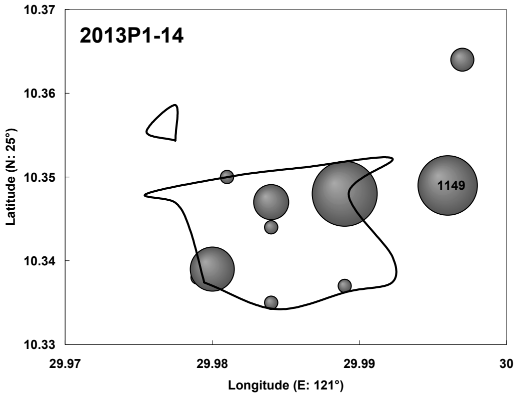
29.99
30
30.01
Longitude (E: 121°)


2013P1-14
1149
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10.35
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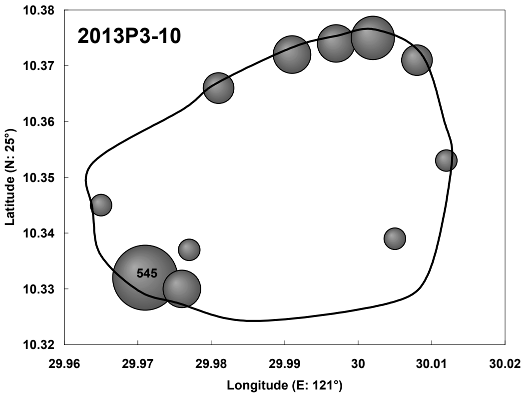
29.99
30
Longitude (E: 121°)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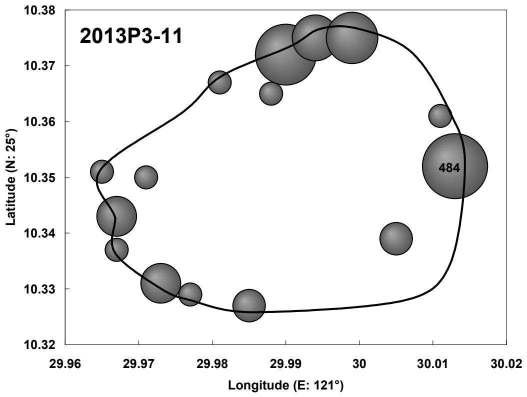
10.37
10.36
10.35
10.34
10.33
10.32
29.96
29.97
29.98
29.99
30
30.01
30.02
Longitude (E: 121°)


2013P3-11
484
Latitude (N: 25°)
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10.37
10.36
10.35
10.34
10.33
10.32
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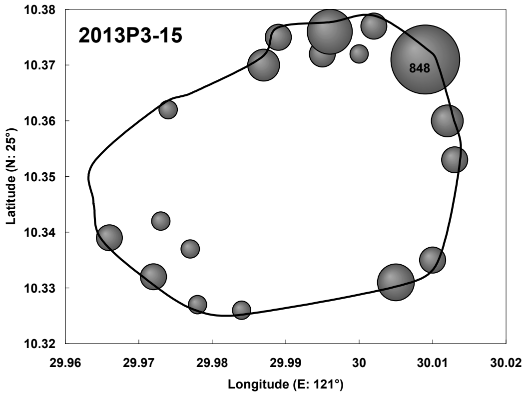
29.97
29.98
29.99
30
30.01
30.02
Longitude (E: 121°)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Longitude (E: 121°)
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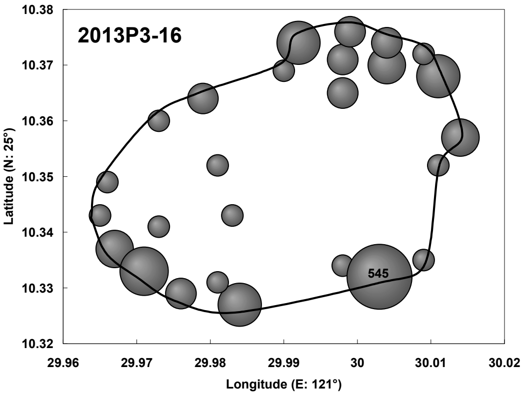
10.32
29.96
29.97
29.98
29.99
30
30.01
30.02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Longitude (E: 121°)
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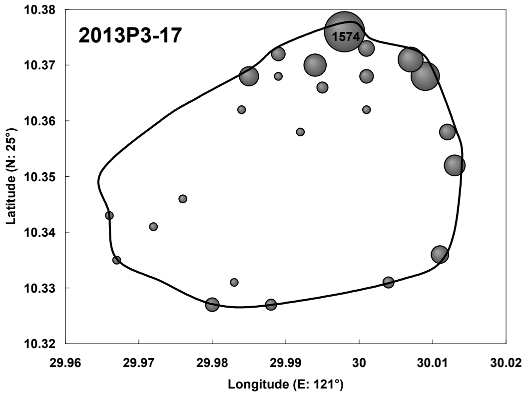
30
30.01
30.02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Longitude (E: 121°)
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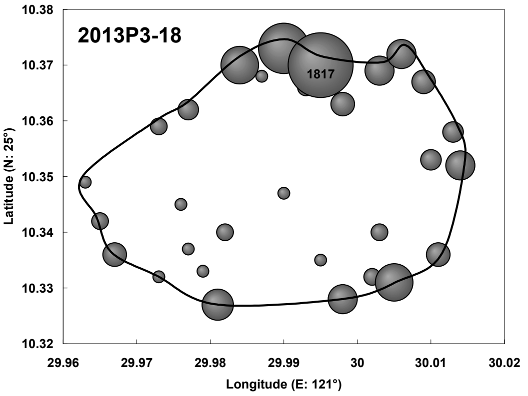
30
30.01
30.02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Longitude (E: 121°)
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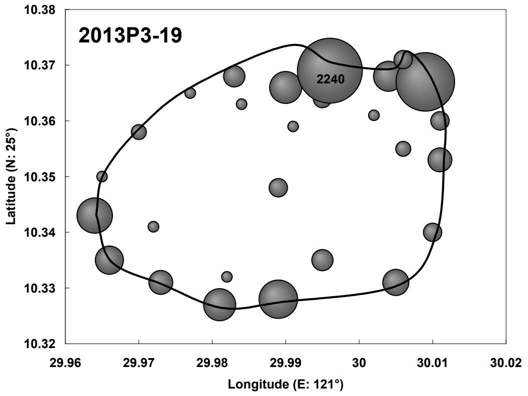
30
30.01
30.02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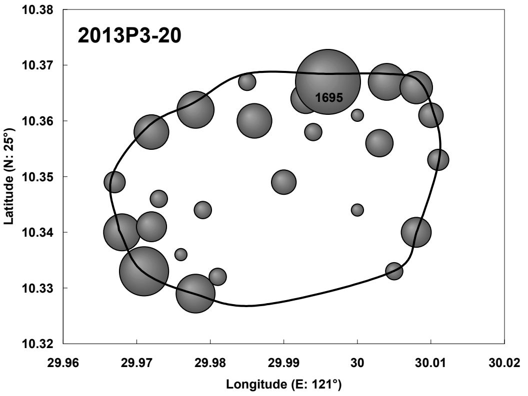
30
30.01
30.02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Longitude (E: 121°)
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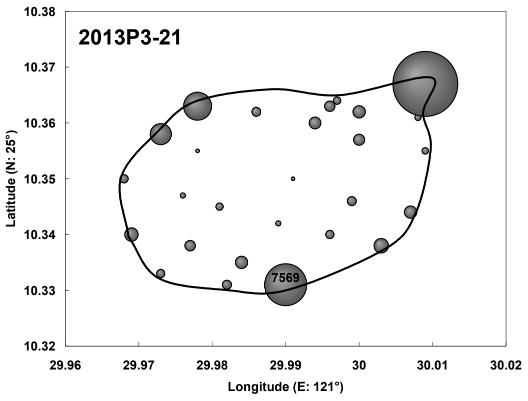
29.98
29.99
30
30.01
30.02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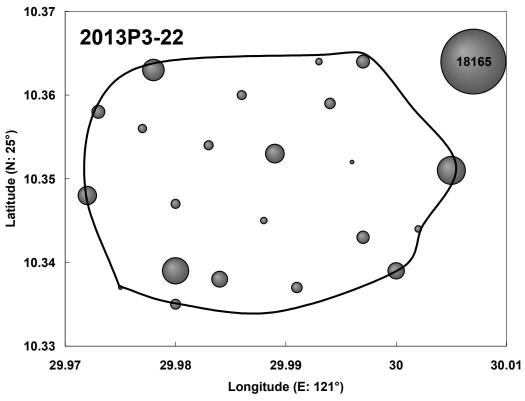
29.98
29.99
30
30.01
30.02
Longitude (E: 121°)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Latitude (N: 25°)
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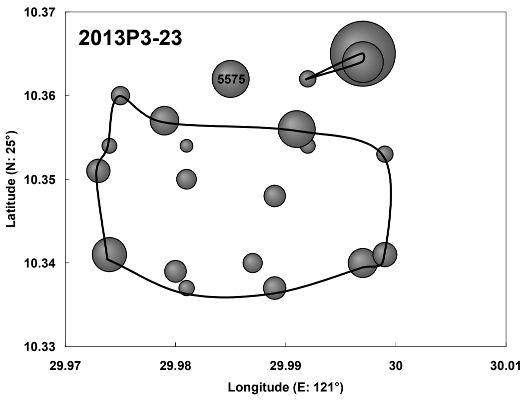
30.01
Longitude (E: 121°)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29.97
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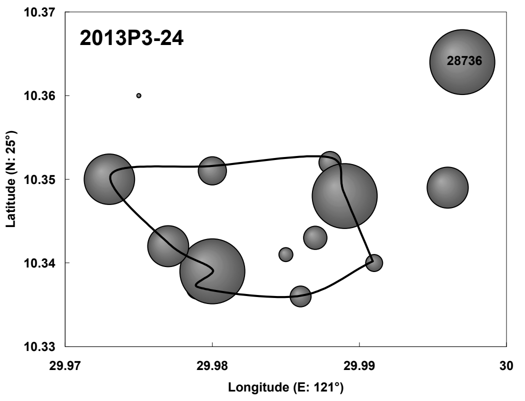
29.99
30
30.01
Longitude (E: 121°)


2013P3-24
28736
Latitude (N: 25°)
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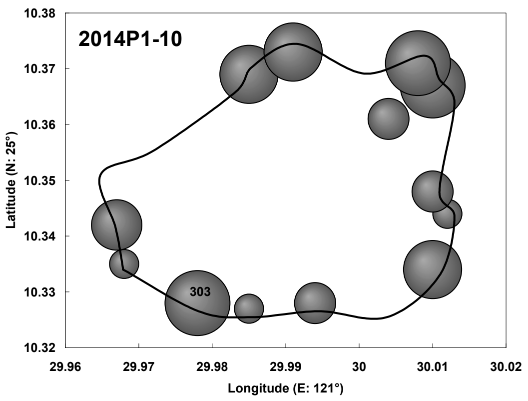
10.36
10.35
10.34
10.33
29.97
29.98
29.99
30
Longitude (E: 121°)


2014P1-10
303
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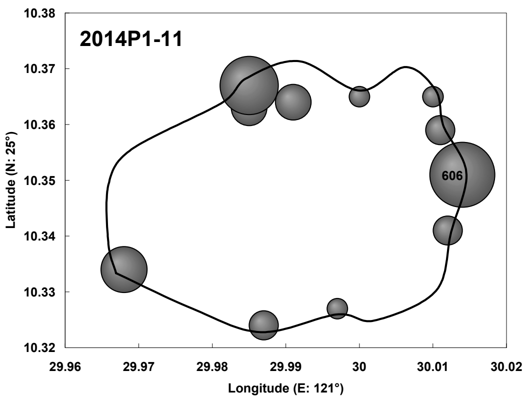
10.37
10.36
10.35
10.34
10.33
10.32
29.96
29.97
29.98
29.99
30
30.01
30.02
Longitude (E: 121°)


2014P1-11
606
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10.37
10.36
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30.01
30.02
Longitude (E: 121°)

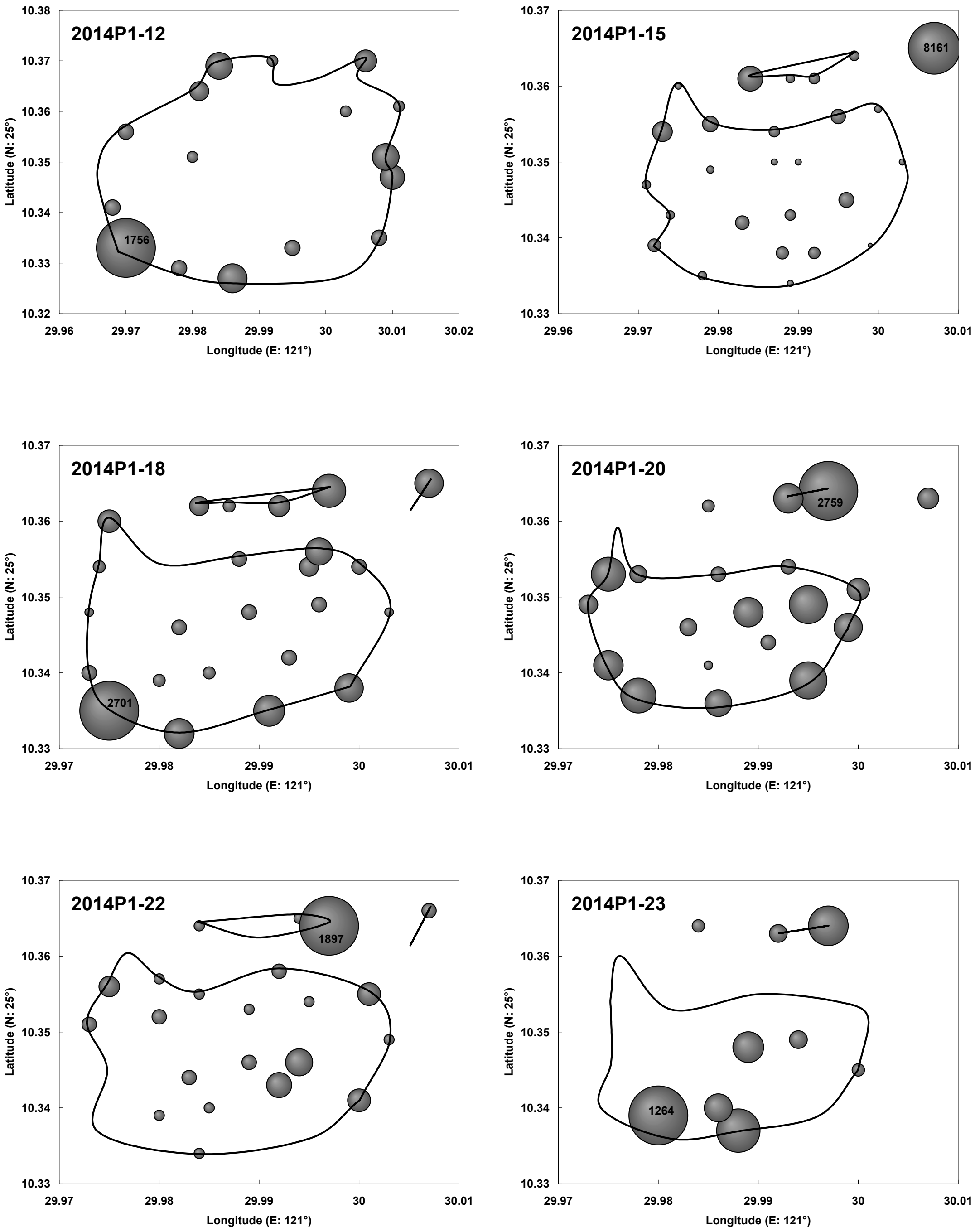
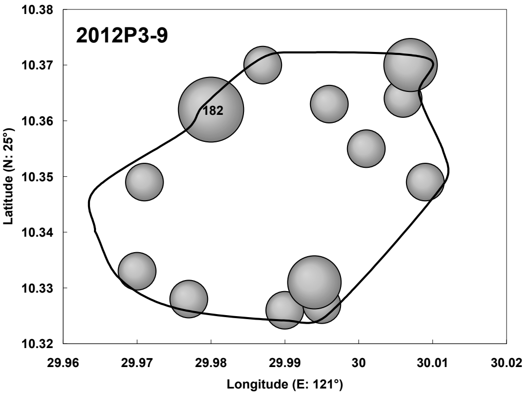


Figure 4. Horizontal distribution of *B. kugenumaensis* through the day. Inundation and time (DAII: days after initial inundation) are marked at the top left corner. The line means pool edge of a given DAII. Numbers in certain balls represent the density ($d_{i,j}$, No./m^2). Be noted that longitude, latitude, and sizes of balls are not consistent

throughout.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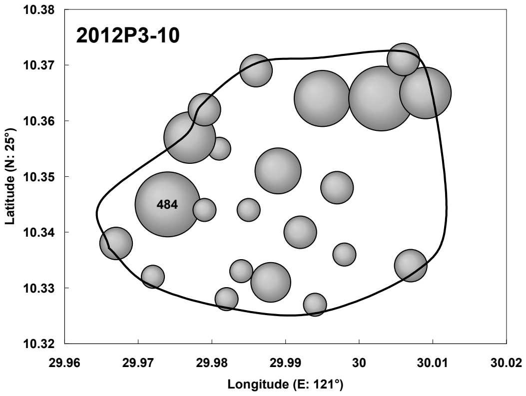
30.01
30.02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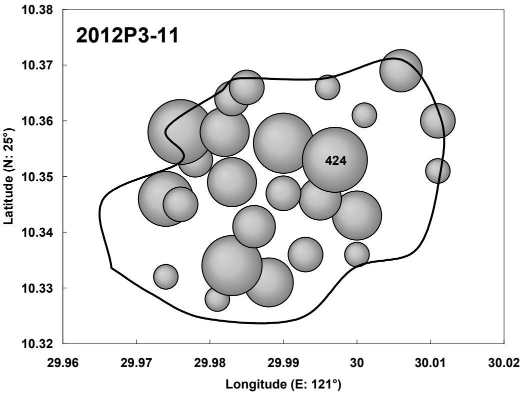
30.02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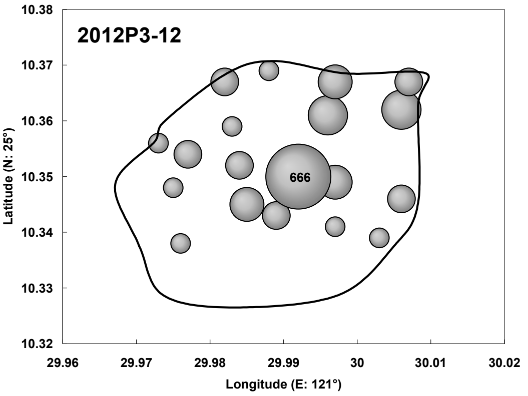
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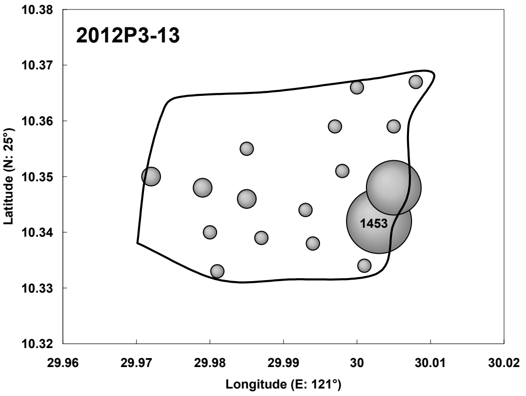
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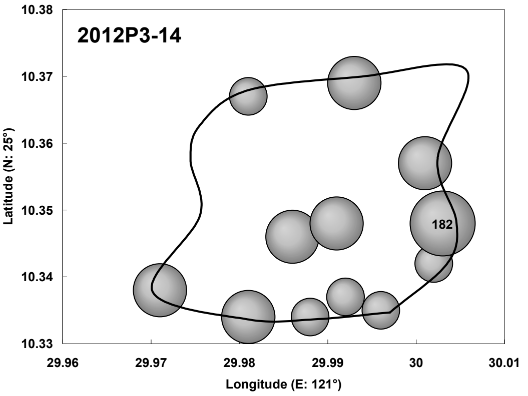
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Longitude (E: 121°)
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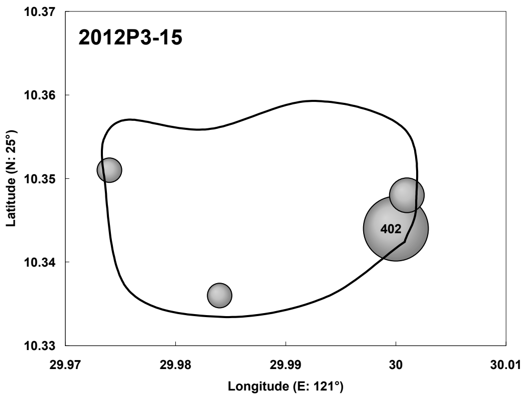
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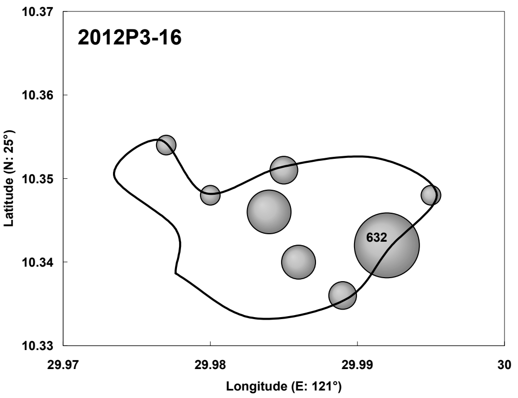
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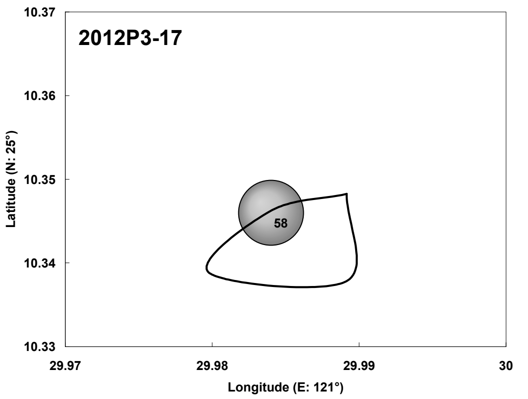
632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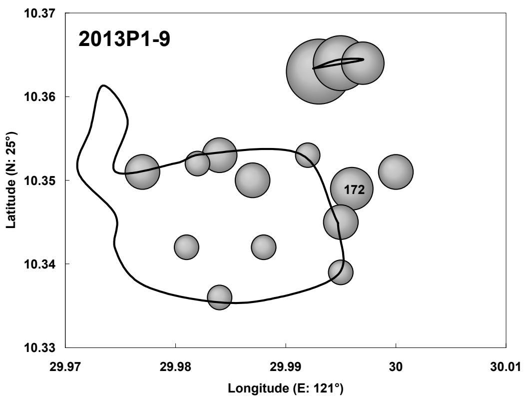
58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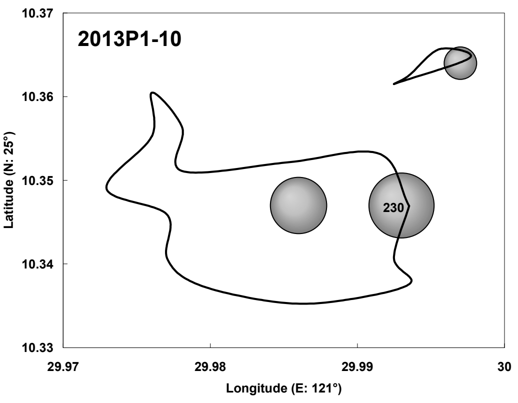
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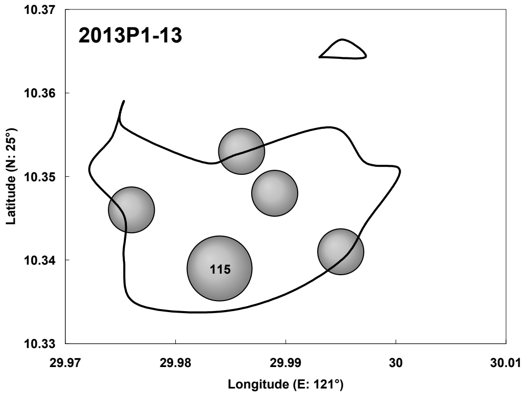
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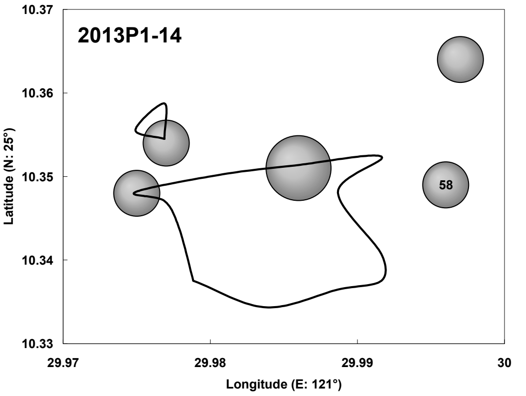
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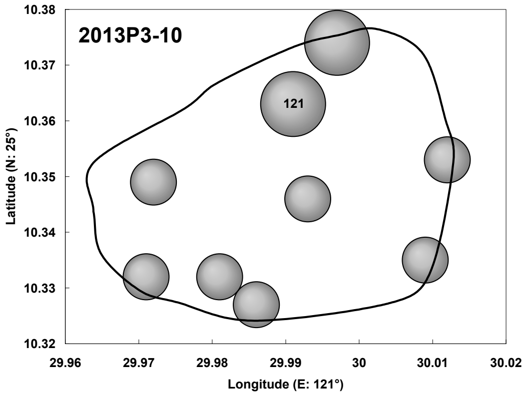
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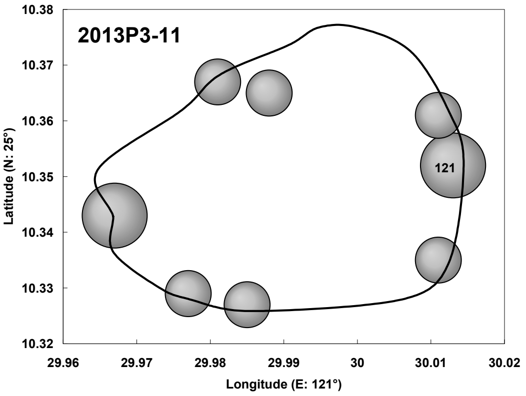
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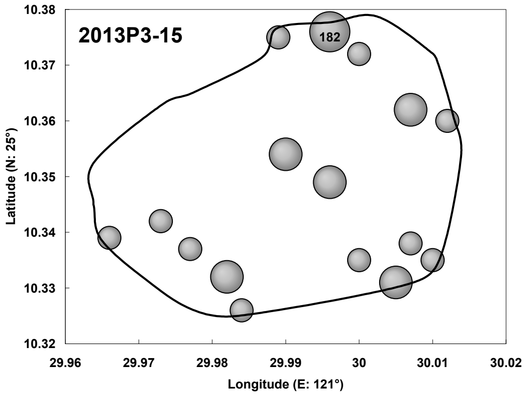
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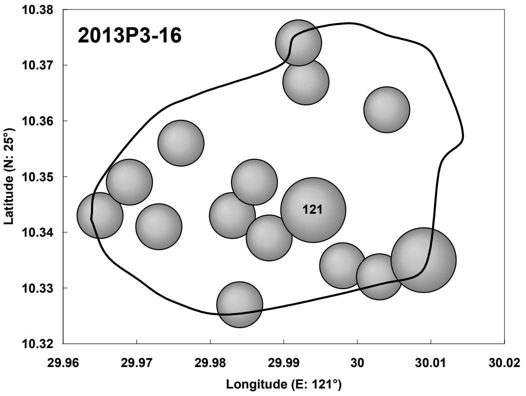
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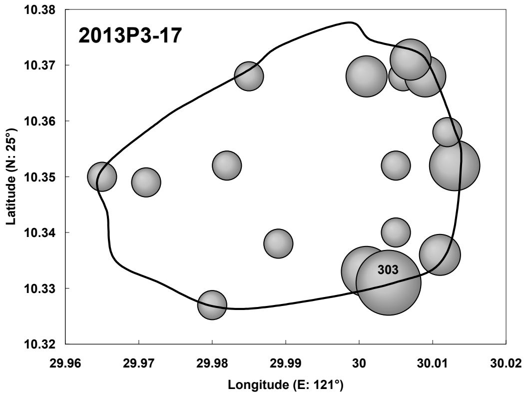
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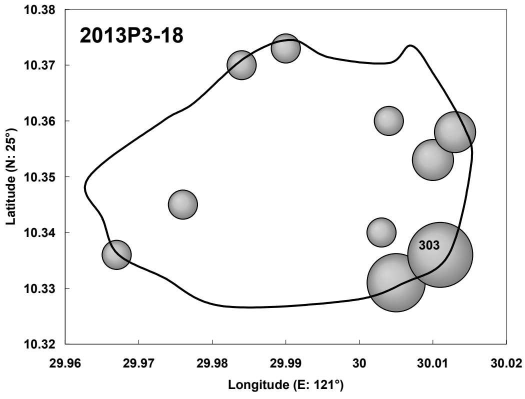
30.01
30.02
Longitude (E: 121°)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30.01
30.02
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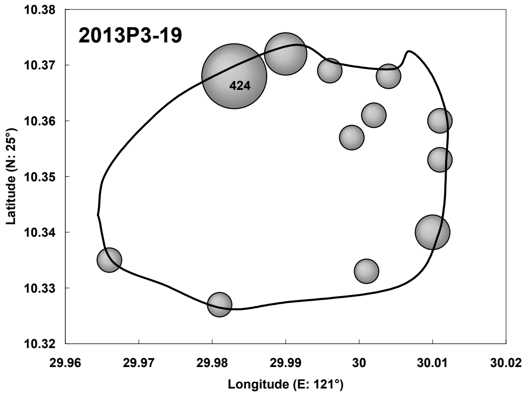
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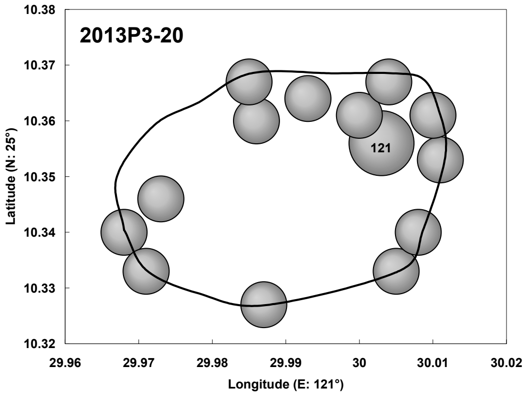
30.01
30.02
Longitude (E: 121°)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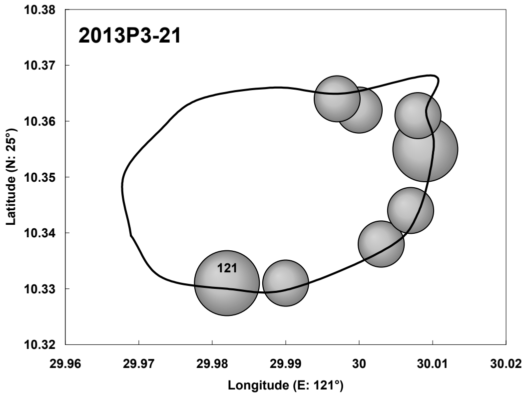
Longitude (E: 121°)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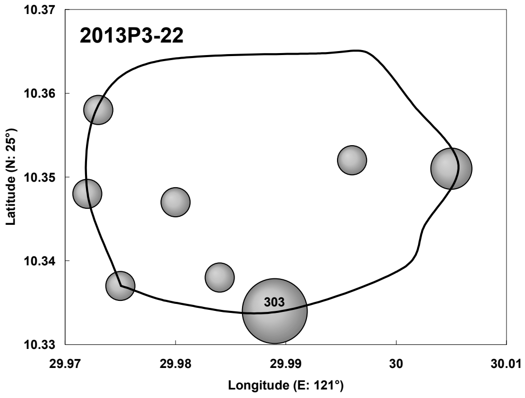
30.02
Longitude (E: 121°)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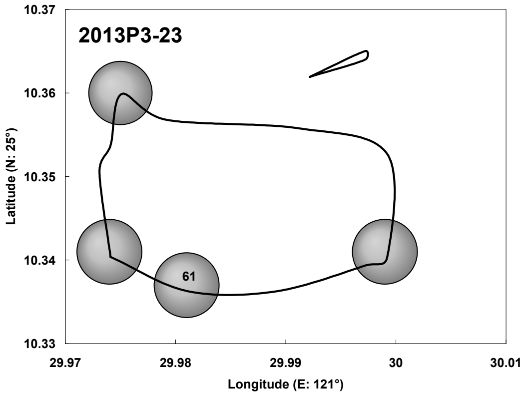
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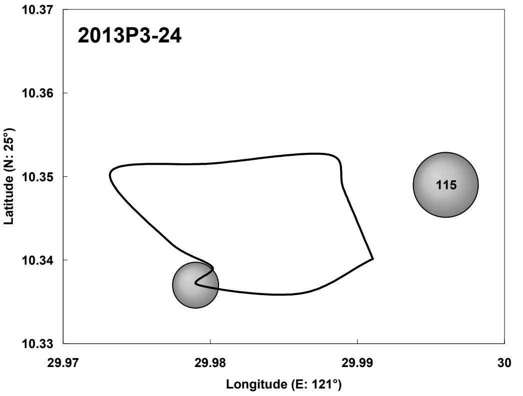
61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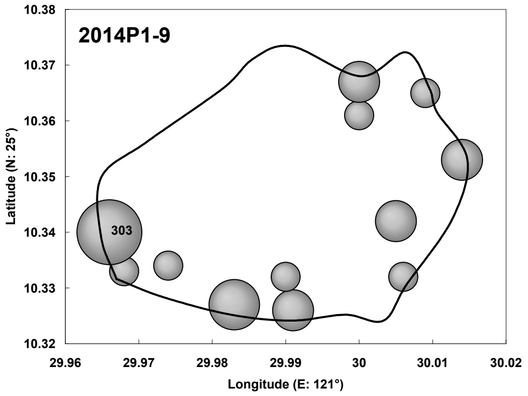
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Longitude (E: 121°)
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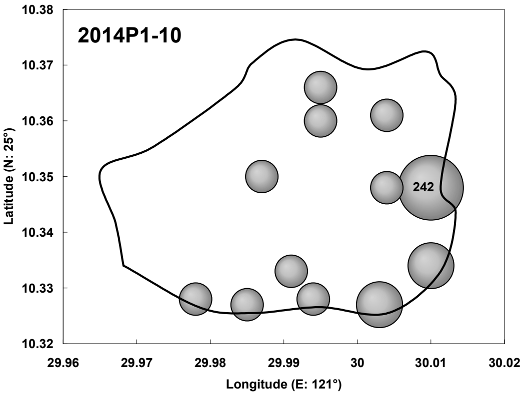
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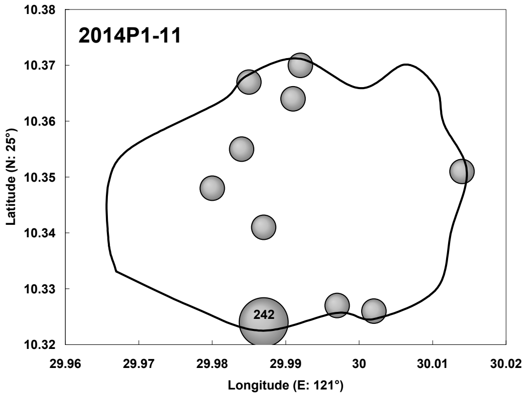
Longitude (E: 121°)
242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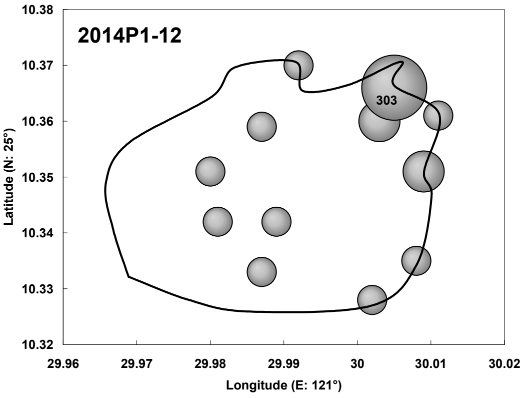
Longitude (E: 121°)
242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Longitude (E: 121°)
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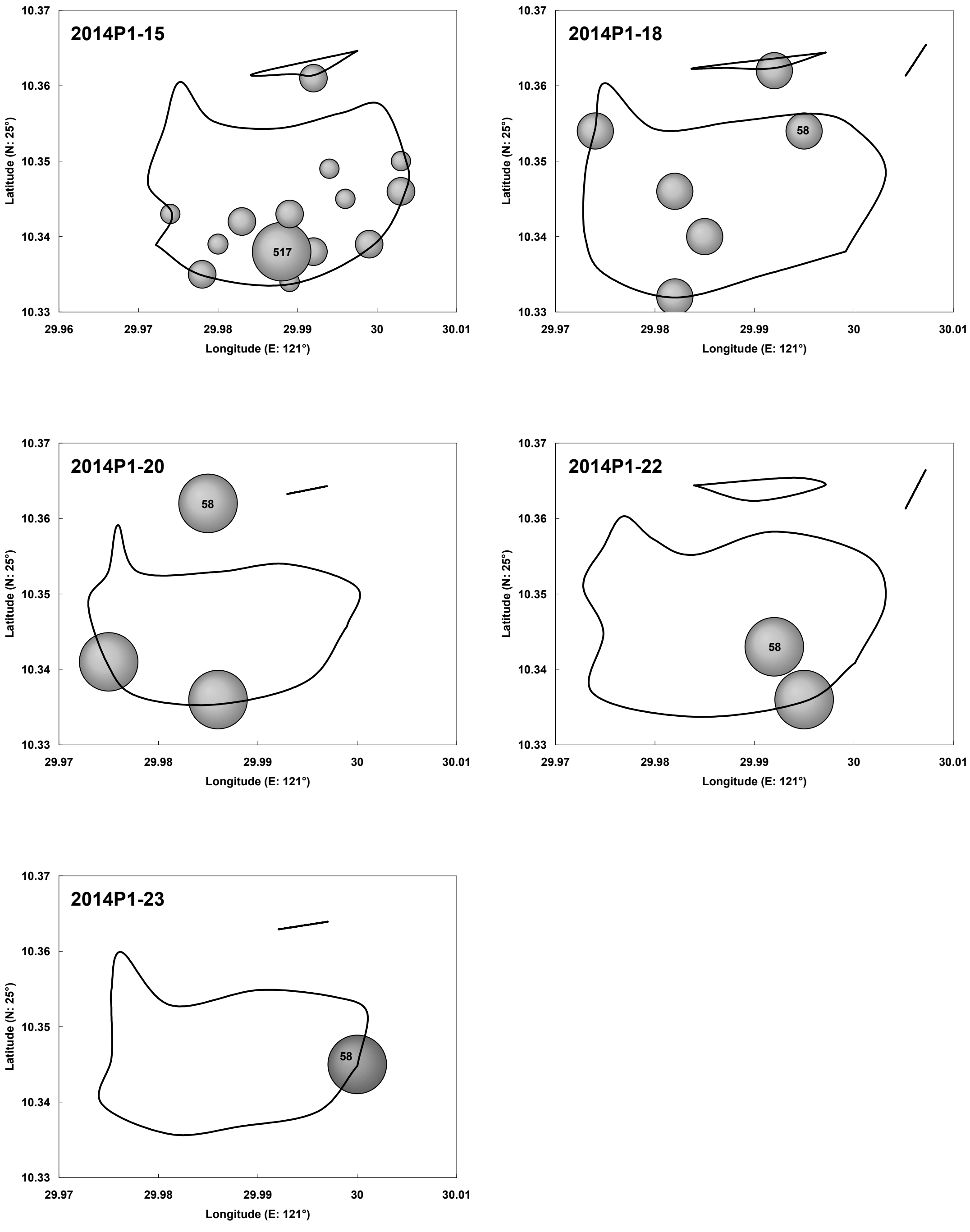
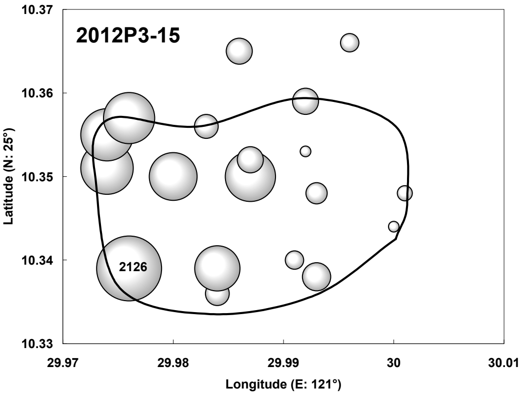


Figure 5. Horizontal distribution pattern of *E. braueriana* through the day. Inundation and time (DAII: days after initial inundation) are marked at the top left corner. The line means pool edge of a given DAII. Numbers in certain balls represent the density ($d_{i,j}$, No./m^2). Be noted that longitude, latitude, and sizes of balls are not consistent

throughout.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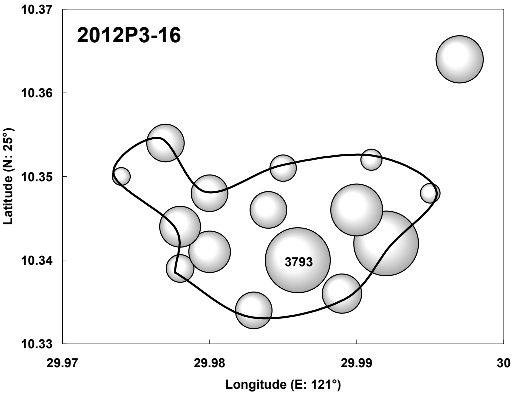
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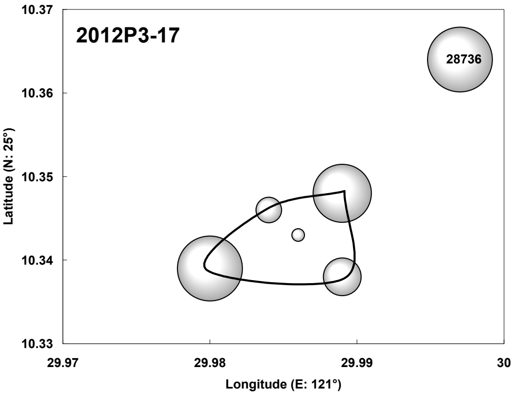
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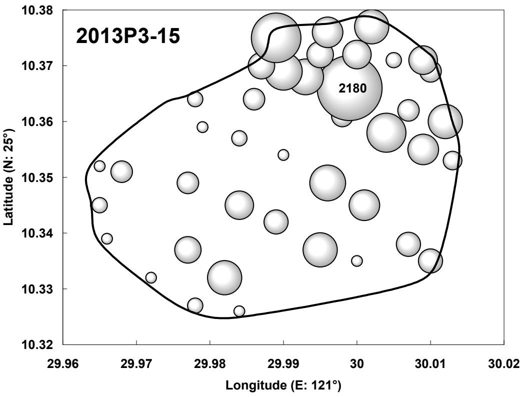
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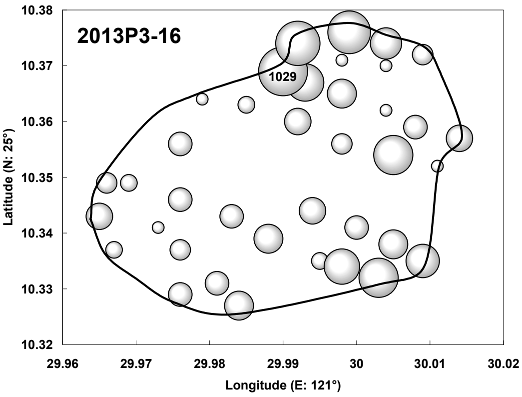
30.01
30.02
Longitude (E: 121°)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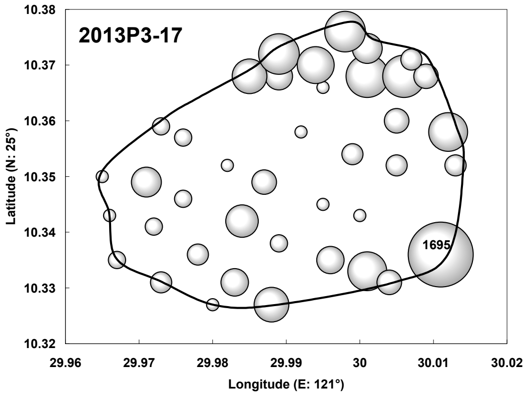
Longitude (E: 121°)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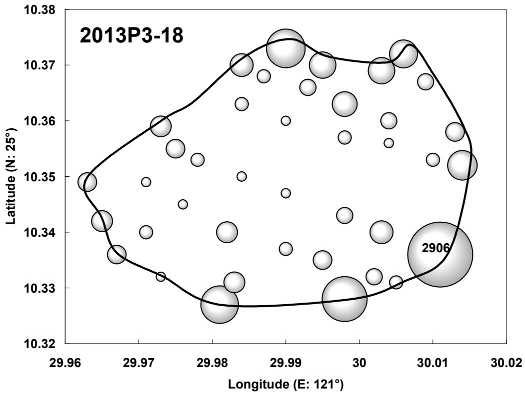
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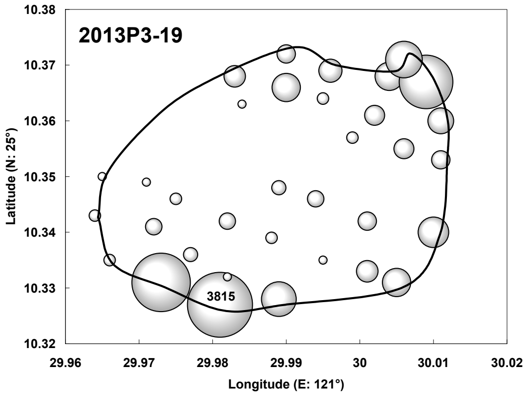
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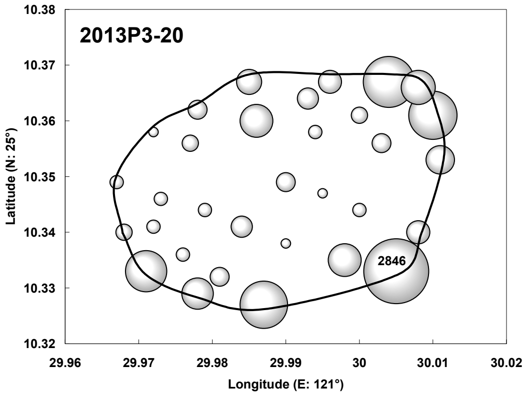
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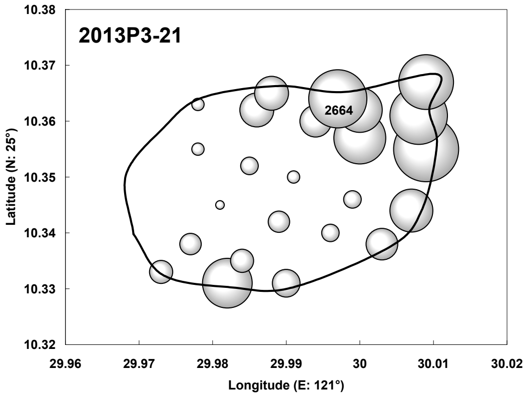
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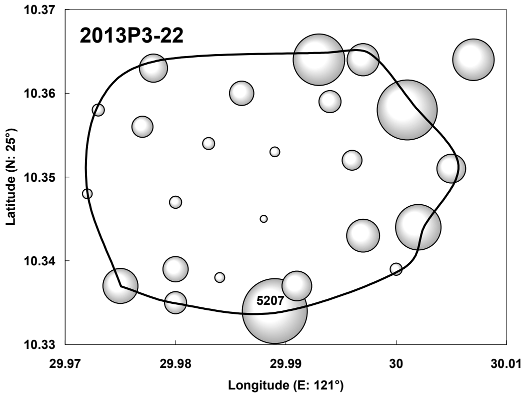
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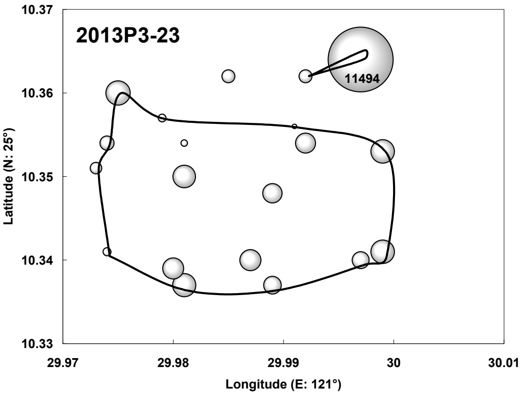
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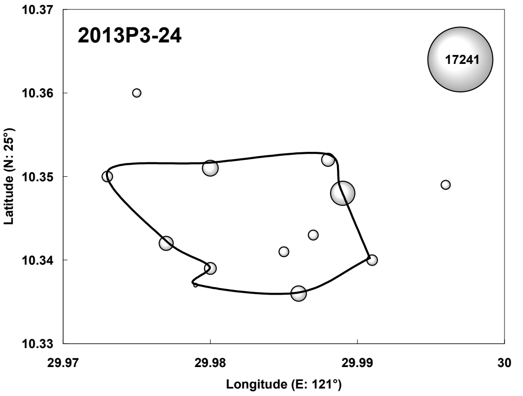
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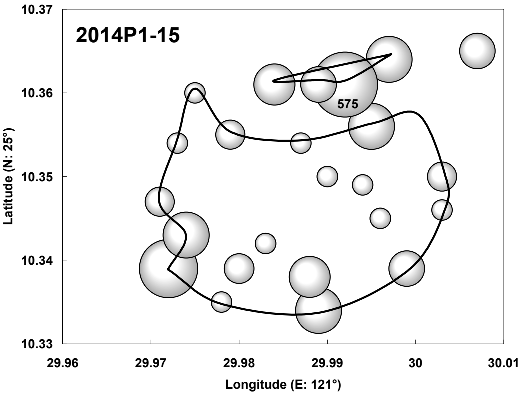
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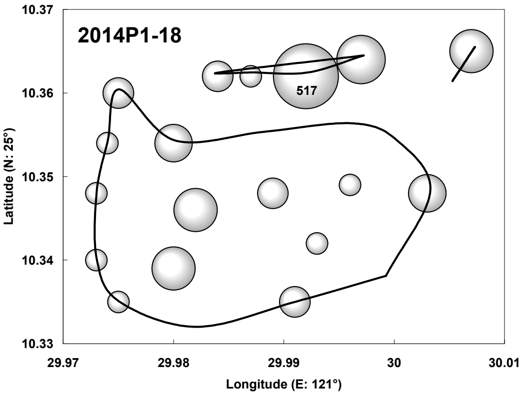
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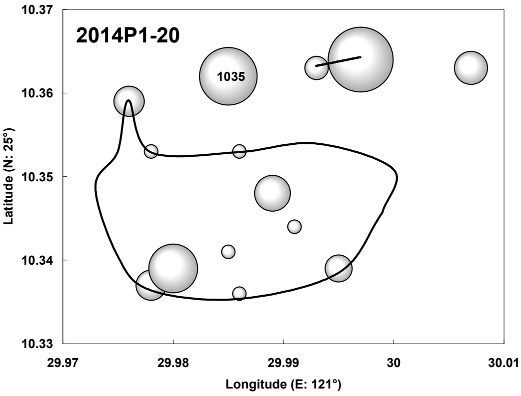
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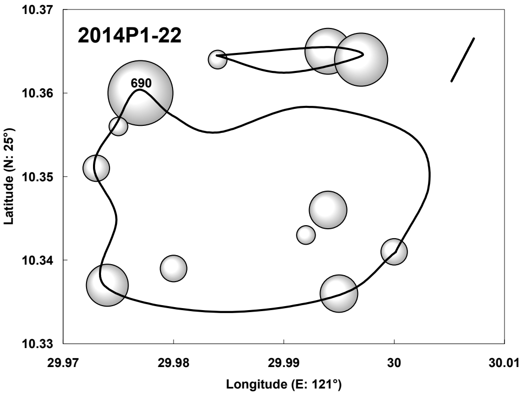
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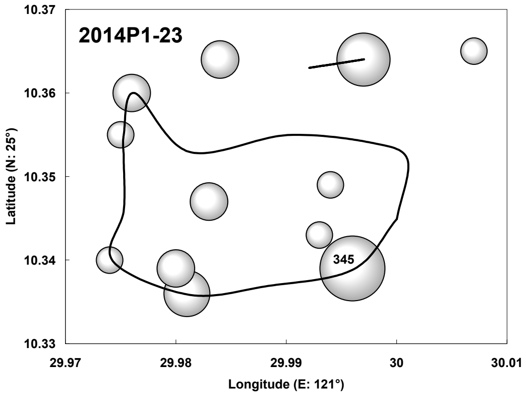

Figure 6. Horizontal distribution pattern of *L. biformis* through the day. Inundation and time (DAII: days after initial inundation) are marked at the top left corner. The line means pool edge of a given DAII. Numbers in certain balls represent the density ($d_{i,j}$, No./m^2). Be noted that longitude, latitude, and sizes of balls are not consistent throughout.

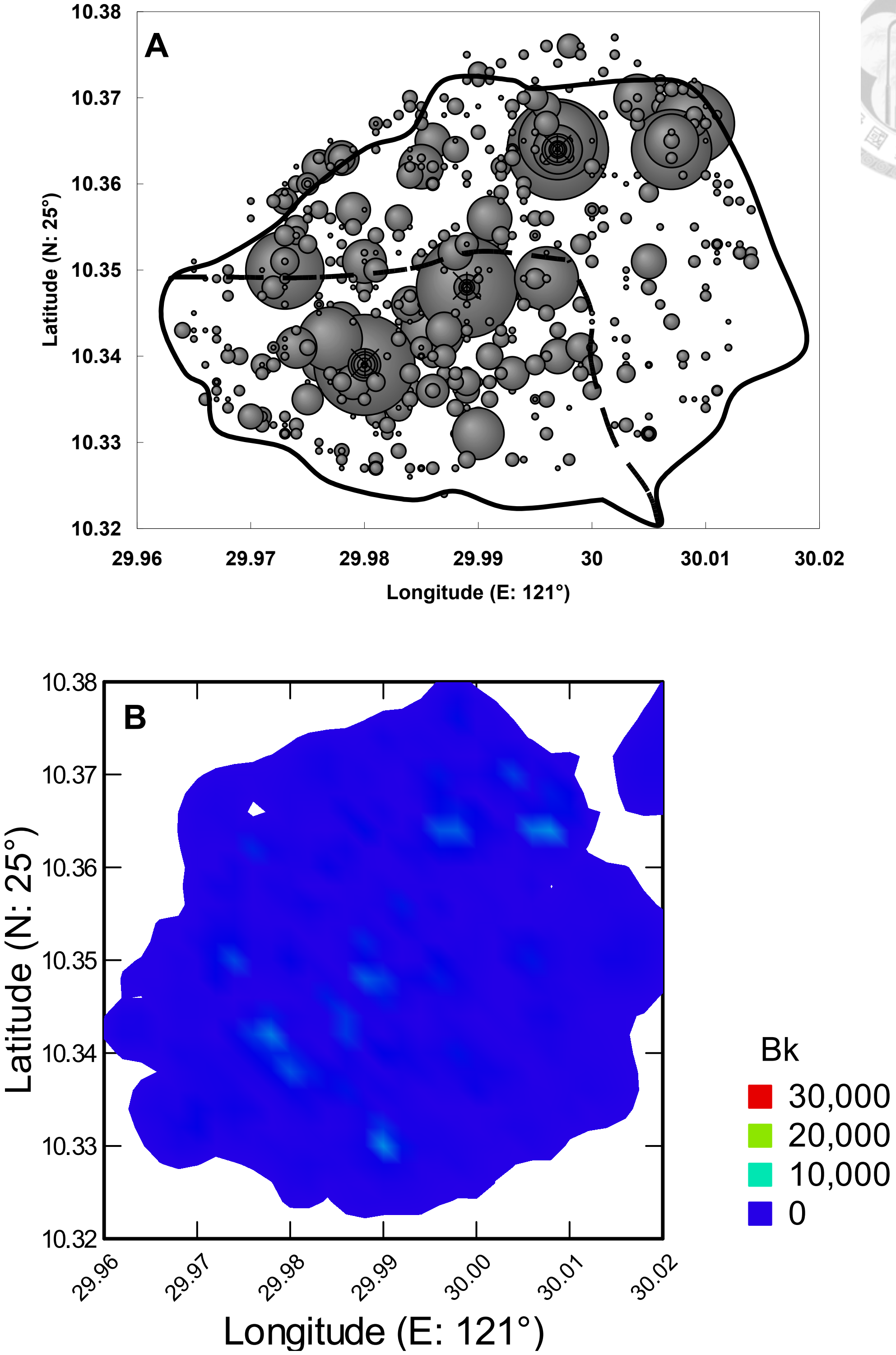

Figure 7. (A) Horizontal distribution throughout the hydroperiods, with characteristic landscapes as in Fig. 1A, and (B) distributional hotspots of *B. kugenumaensis*.

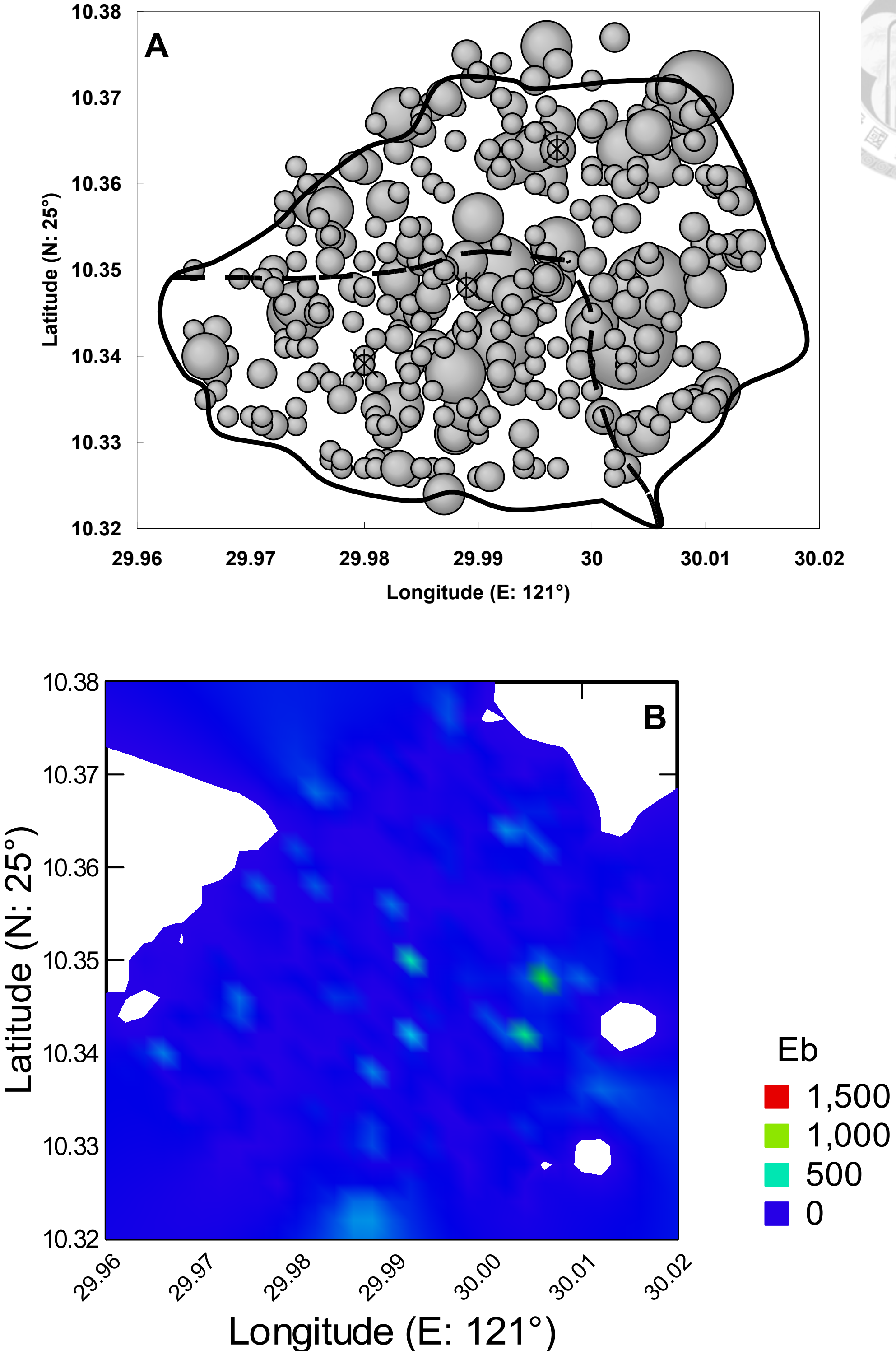

Figure 8. (A) Horizontal distribution throughout the hydroperiods, with characteristic landscapes as in Fig. 1A, and (B) distributional hotspots of *E. braueriana*.

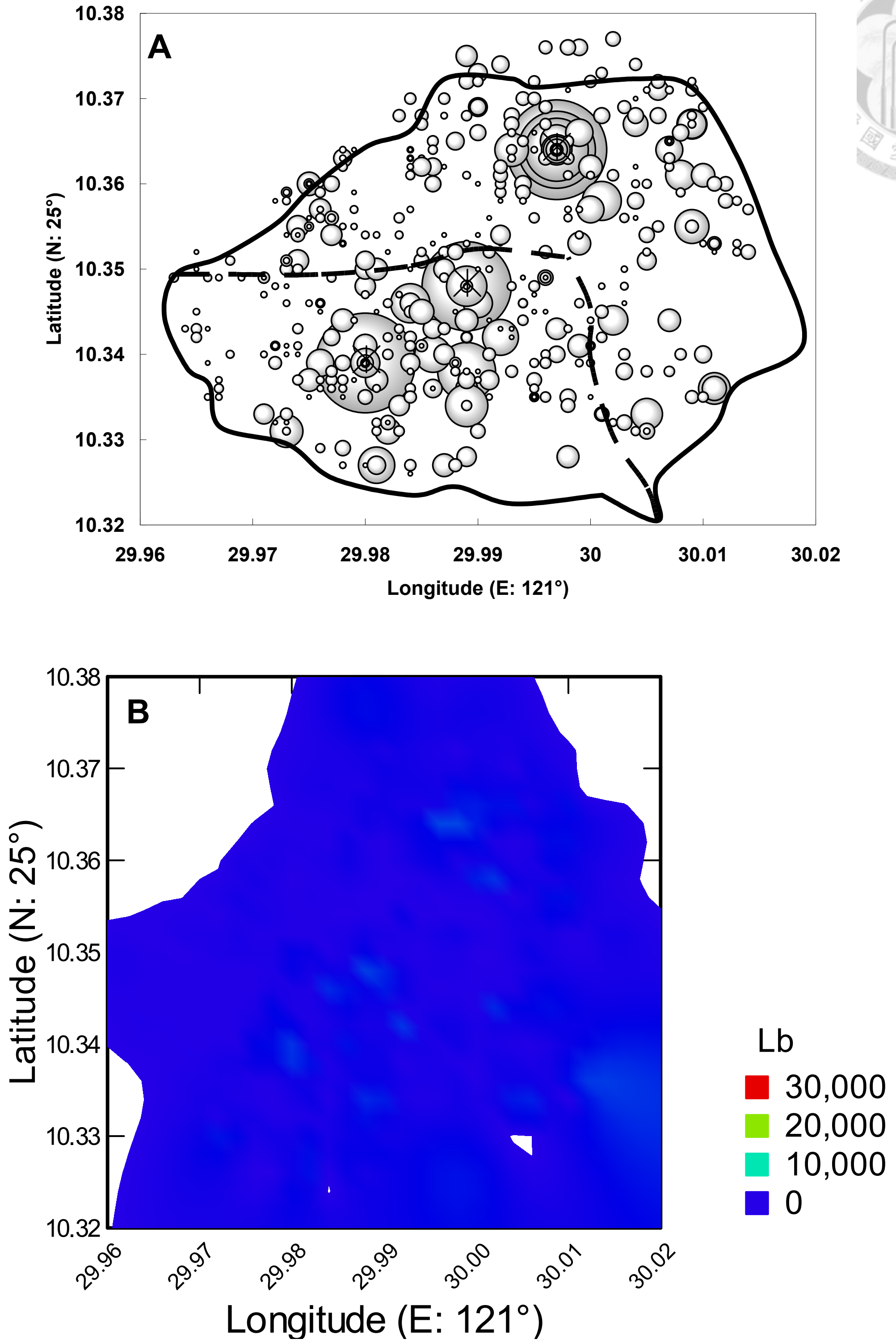

Figure 9. (A) Horizontal distribution throughout the hydroperiods, with characteristic landscapes as in Fig. 1A, and (B) distributional hotspots of *L. biformis*.

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Chapter 3: Egg bank spatial structure and functional size of three sympatric branchiopods (Branchiopoda) in Siangtian Pond, Taiwan

Abstract

Large branchiopods in temporary wetlands produce eggs that hatch in an asynchronous sequence to overcome stochastic hydroperiods. Long-term dynamics between egg replenishment and depletion result in habitat-specific egg bank spatial structures. Unlike earlier studies focusing mainly on the active stages, this study explores the horizontal distributions and functional sizes of the egg banks of three large branchiopods, *Branchinella kugenumaensis, Eulimnadia braueriana*, and *Lynceus biformis*, in Siangtian Pond, Taiwan. Superficial sediment was systematically collected from 58 sites in a defined “core area,” and eggs were identified and quantified in 0.5 g sediment aliquots, with ten replicates for each site. The egg density was calculated to estimate the egg bank size and was plotted to show the spatial distribution patterns. I found that of the three species, *L. biformis* was the most abundant in the egg bank, while the egg bank size of *B. kugenumaensis* was close to cohort fecundity, indicating limited egg accumulation. Horizontal distributions varied significantly between species, with *B. kugenumaensis* concentrated centrally at the three deepest sites, *E. braueriana* aggregated peripherally in less vegetated areas, and *L. biformis* found predominately in the south. The egg bank spatial structure could be partially explained by the varying inundation area and population maturation times. However, egg floatation and wind action may also be influential.

Key words: *Branchinella kugenumaensis*, dormant egg, *Eulimnadia braueriana*, large branchiopods, *Lynceus biformis*

Introduction

Large branchiopods (Branchiopoda: Anostraca, Notostraca, Laevicaudata, Spinicaudata) inhabit temporary wetlands, which undergo frequent and recurrent changes between favorable and unfavorable periods (Hildrew, 1985; Williams, 1985; Brendonck et al., 2008). Several strategies have evolved to deal with this environmental stochasticity, including rapid growth and maturation, and dormant egg production (Hildrew, 1985; Brendonck, 1996; Brendonck et al., 1998, 2000; Vanschoenwinkel et al., 2010). Specifically, drought resistant dormant eggs are necessary to bridge adverse conditions, acting as a biological reservoir (Brendonck, 1996; Fryer, 1996; Hairston, 1996; Brendonck and De Meester, 2003).

Branchiopod dormant eggs from one cohort or brood, however, do not generally hatch all at once during a single hydroperiod, with hatching spread instead over several hydroperiods. This phenomenon of partial and gradual hatching has been noted as a bet-hedging strategy (Saiah and Perrin, 1990; Simovich and Hathaway, 1997; Mura, 2001; Brendonck and De Meester, 2003; Ripley et al., 2004; Maffei et al., 2005) used to avoid the incidence of abortive hatching, where the population crashes as all the eggs hatch but are unable to reach sexual maturity before the pool dries out (Brendonck, 1996). Consequently dormant eggs accumulate to form an egg bank with overlapping generations in the sediment (Hairston, 1996; Brendonck and De Meester, 2003).

The spatial distribution of dormant eggs in a temporary pool is chiefly shaped by local population dynamics over the long-term history of the given habitat. Factors such as the location, time, and density of egg production, hatch depletion, senescence, mortality, and passive dispersal, can affect the egg bank spatial structure (Thiéry, 1997; Brendonck and De Meester, 2003). Environmental and additional biological factors, such as hydroregime and life history, can lead to habitat-specific patterns in egg bank distribution, especially when multiple species coexist with diverse life history strategies and hatching phenologies (Cáceres, 1997; Brendonck and De Meester, 2003; Gyllström and Hansson, 2004).

Previous large branchiopod studies at Siangtian Pond in northern Taiwan have demonstrated the occurrence of indeterminate growth patterns, developmental schedules (Huang et al., 2010), and fecundity (Huang et al., 2011) in the fairy shrimp *Branchinella kugenumaensis* (Ishikawa, 1895). Moreover, a recent study has shown significant spatial differentiation in free-living individuals of the three sympatric branchiopod species, including *B. kugenumaensis* and two species of clam shrimp, *Eulimnadia braueriana* Ishikawa, 1895 and *Lynceus biformis* (Ishikawa, 1895), in Siangtian Pond (Wang et al., 2012). Although the active stages have been studied systematically, the dynamics of the dormant stage of this large branchiopod community remain unknown.

It has been pointed out in different case studies, however, that egg bank spatial structures could be influenced by the distribution patterns of the free-living stage. De Stasio (1989) demonstrated a significant link between active population and egg distribution patterns of freshwater copepod *Diaptomus sanguineus* Forbes, 1876, as indicated by both higher densities of females and a greater abundance of eggs in the central regions of the study area. Thiéry (1997) also suggested that peripherally distributed eggs of *Triops numidicus* (Grube, 1865) could be explained by female egg-laying preferences. Since Siangtian Pond is characterized by a rapid but stable water level reduction rate (32 cm/day; Huang et al., 2010), microhabitats available for the inhabiting large branchiopods also change accordingly, possibly imposing strong limitations on spatial utilization by active populations. In this scenario, I assumed that the spatial distribution pattern of spawning adults would determine the spatial structure of the egg bank, and I hypothesized that the horizontal egg bank distribution could be related to the population maturation time. Based on this hypothesis, I predicted that the earlier the species spawned, the more widely the eggs would be scattered over the pond. Conversely, the later the species spawned, the closer the eggs would be aggregated towards the center. If, however, this hypothesis was rejected, factors other than branchiopod maturation and water reduction should be considered to explain the egg bank spatial structure. In this study, I focused on the functional size of the egg bank and the egg bank spatial distribution of the sympatric *B. kugenumaensis*, *E. braueriana*, and *L. biformis* in Siangtian Pond, considering biological as well as environmental factors contributing to its habitat-specific spatial structure.

Materials and Methods

Study site

This study was conducted in Siangtian Pond of Yang-Ming-Shan National Park in northern Taiwan. As described in Huang et al. (2010, 2011) and Wang et al. (2012), this seasonally astatic wetland typically fills during monsoonal rains or typhoons, covering an area of 10^4 m^2 and reaching a maximum depth of 6 m. Without replenishing rainfall, the water level decreases at a stable rate of 32 cm per day, and the hydroperiod lasts for only 6-17 days. Occasionally, prolonged hydroperiods occurred, e.g. lasting for 32 and 50 days in 2004 and 2008 respectively (Huang et al., 2010). With regard to vegetation, the pool is dominated in the center by common rush (*Juncus effusus* Lazenby, 1955) and around the margins by maiden grass (*Miscanthus sinensis* Andersson, 1855). This ephemeral habitat supports three species of large branchiopod crustaceans: *B. kugenumaensis*, *E. braueriana*, and *L. biformis*.

Field Survey and Sampling

Sampling was conducted from 17-19 May 2010 during a dry period. Sampling points were confined to the commonly inundated area of a 3 m isobath, although the maximal inundation depth can reach more than 4 m, even occasionally exceeding 6 m (Huang et al., 2010). This particular range was selected as the “core area” for sampling because it is typically inundated consistently for the same period of time that *B. kugenumaensis* requires for spawning the first brood (Huang et al., 2010; Fig. 1A, with an airscape of the dry pool in Fig. 1B). However, there was a sampling gap between the human-made path and a small, depressed area towards the northern tip of the study site (Fig. 1A) due to a ridge that divides the water body into two separate parts during the later period of the 2 m isobath. This ridge area was therefore excluded from sampling. I used a five-meter grid for determining sampling points. At each sampling point, I took the superficial sediment layer where most viable eggs generally occur (Marcus et al., 1994; Brendonck and De Meester, 2003; Mura, 2005; Dahms et al., 2006; Hulsmans et al., 2006). The dimensions of each aliquot were 20 cm × 20 cm × 5 cm (length × width × depth) and the GPS coordinates of each sampling point were recorded (Garmin®, Dakota 20). A total of 58 points were collected, covering 2.32 m^2 of the surface area, approximately 0.03% of the total area of the pool when it is 3 m deep (7177.2 m^2 as calculated in Huang et al., 2011).

I also measured the vegetative coverage (C_s; %) and vegetation height (H_s; cm) within a 900 cm^2 square around each sampling point (s: the s^{th} sampling point). I took a photo of each square to calculate C_s. The photo was input into the software ImageJ, which defined the whole sampled area and calculated the coverage proportion of the vegetation (C_s). To calculate H_s, each square was equally divided into four 225 cm^2 quadrants. I defined height (H) as the natural standing height of the vegetation above the substrate and measured it at the apex of each quadrant. If H was more than 100 cm, it was treated as 100 cm. I performed a total of nine measurements to obtain the mean H_s for each sampling point.

Egg Identification and Enumeration

Sediment from each sampling point was air dried at room temperature (25-27 °C), and the evident vegetation was removed. The samples were then weighed and stored in the dark at room temperature. The eggs of *B. kugenumaensis*, *E. braueriana*, and *L. biformis* were identified by their distinct external morphologies (Fig. 2) under a stereomicroscope (Leica®, MZ 125), using comparative material taken previously from egg-laden adults from the same site.

Dried sediment typically contained clumps in a broad range of sizes from several millimeters to several centimeters, with the number of clumps differing across different sampling points. These clumps mostly remained intact despite being immersing in distilled water or aged tap water, and even in a saturated sodium chloride solution or a saccharose solution (sugar flotation method, 1000 g saccharose dissolved in 1000 g distilled water; Onbé, 1978; Vandekerkhove et al., 2004a). These dirt clumps may be lumped by fine and dense roots or sticky substrate, and thus may have been protected from precipitation disturbance in Siangtian Pond by the vegetation above. This phenomenon was suggested to have helped stabilize the egg bank (Vanschoenwinkel et al., 2010). For this reason, I did not try to artificially break the larger sediment clumps by way of sonication (Vandekerkhove et al., 2004b) but directly counted all eggs that were at least partially exposed. After evenly mixing the substance of each soil sample and discarding the obvious clumps (e.g. those ≥ 2 mm in diameter), 0.5 g of sediment was randomly sampled, while all exposed eggs were counted and identified according to species. Although this method may underestimate the actual egg count by ignoring eggs inside the clumps, this underestimation was reduced to a very minor scale since my estimate referred to the functional (viable) egg bank size. Only exposed eggs were assumed to be viable and constituted the functional egg bank because they could be in direct contact with the aquatic surroundings and would logically have the highest opportunity to hatch and contribute to the free-living population. In contrast, those eggs buried inside the soil clumps would be least likely to receive the environmental signals needed to hatch, greatly reducing their hatching potential during the hydroperiod

(Gyllström and Hansson, 2004). This process of 0.5 g soil sampling and egg counting was repeated ten times for each sampling point. In all, a total of 5.0 g of sediment was investigated for each sampling point.

Egg Bank Spatial Structure and Functional Size Evaluation

Mean (± standard deviation) egg number for each branchiopod species per 0.5 g sediment in each sampling site, $e_{b,s}$ (in which b represents branchiopod species ($b = 1, 2, 3$) and s the s^{th} sampling point) was calculated from the resampled data by Monte Carlo random sampling (SYSTAT® 12) from the ten originally examined subsamples after 500 iterations. Since I investigated only 5.0 g of sediment for each sampling point, the effect of extreme values may incline the mean and enlarge the standard deviation in small sample sizes. Generating 500 resampled values can reduce the amount of extreme values in data and lead to a confident mean with a more convergent standard deviation. In addition, the dirt samples from sampling points were not of the same weight due to containing different ratios of water and vegetation. Therefore, the unit of egg density by weight (2 x $e_{b,s}$, egg number per gram) was transformed into egg density by area ($d_{b,s}$, egg number per m^2) by

$$d_{b,s} = \frac{2 \times e_{b,s} \times w_s}{0.04} \quad (1),$$

where w_s represents the weight of the total sampled dirt at the s^{th} sampling point. The area of each sediment sampling point was 400 cm^2, or 0.04 m^2. Then, the functional egg bank size of each species (E_b) was calculated by

$$\overline{D_b} = \frac{\sum_{s=1}^{58} d_{b,s}}{58} \quad (2),$$

$$E_b = \overline{D_b} \times A \quad (3),$$

in which $\overline{D_b}$ and A represent the mean egg density per m^2 of the branchiopod species and the total area enclosed by the boundary of the inundated area with a 3 m isobath (equaling to 7177.2 m^2; Huang et al., 2011), respectively.

Horizontal Distribution Transformation into Linear Distance

To test whether $d_{b,s}$ showed a linear correlation with the distance from the morphometric center (c_1) of Siangtian Pond, the distance of the s^{th} sampling point to c_1 (D_s; m) was calculated by

$$D_s = \sqrt{[(x_s - x_{c_1}) \times 1850]^2 + [(y_s - y_{c_1}) \times 1750]^2} \quad (4),$$

where x_s and y_s represent the longitudinal and latitudinal coordinates of the s^{th} sampling point respectively, and x_{c_1} and y_{c_1} are the longitudinal and latitudinal coordinates of c_1, respectively. In Equation 4, the 1850 and 1750 are the length (m) of a minute of longitude and latitude, respectively. Since Siangtian Pond has three local maximum depths, I transformed the distance of the s^{th} sampling point according to these three points (D_s'; m) by

$$D_s' = \frac{D_{sc_1} + D_{sc_2} + D_{sc_3}}{3} \quad (5),$$

in which D_{sc_1}, D_{sc_2}, and D_{sc_3} represent the distance of the s^{th} sampling point to the deepest place (c_1), 2nd deepest place (c_2), and 3rd deepest place (c_3), respectively. In this scenario, I defined all three deepest points as the distribution center ($D_s' = 0$).

Maturation Time Determination

Field collection for the free-living stage of this large branchiopod community was conducted whenever Siangtian Pond was inundated from 2004 till 2013, except 2009. 30-60 individuals of each species were sampled randomly by plankton net and brought back to the laboratory for life history studies (Huang et al., 2010; 2011). Females of *B. kugenumaensis* and *L. biformis*, and hermaphrodites of *E. braueriana* were examined under a stereomicroscope (Leica®, MZ 125). I defined the maturation time (*ts*; days after initial inundation, *DAII*) of the population to be the number of days until more than 50 % of the females/hermaphrodites had well enveloped eggs in the brood pouch (*B. kugenumaensis*) or dorsal side (*E. braueriana* and *L. biformis*), the same definition as the "time at first spawning" in Huang et al. (2010). *Branchinella kugenumaensis* was the first to be monitored in 2004, and records were kept throughout all subsequent periods, but no detailed data was gathered on either *E. braueriana* or *L. biformis* until 2006 and 2008, respectively.

Data Analysis

All the C_s, H_s, and $d_{b,s}$ were analyzed by two-dimensional contour mapping (SYSTAT® 12) to clarify the spatial distribution patterns of egg bank spatial structures. The correlation between the log-transformed $d_{b,s}$ and D_s / D_s' were tested by linear regression.

Results

The vegetation coverage (C_s) was almost zero around the man-made path, but increased rapidly towards other regions (Fig. 3A). The vegetation height (H_s) was also the lowest around the path, increased gradually towards the southern common rush region, and reached more than 1 m near the periphery that was mainly vegetated by maiden grass (Fig. 3B).

The egg densities ($\overline{D_b}$) and egg bank sizes (E_b) of *B. kugenumaensis*, *E. braueriana*, and *L. biformis* are presented in Table 1, with approximately 30,000 eggs per m^2 for *B. kugenumaensis* and *E. braueriana*, and 241,184 eggs/m^2 for *L. biformis*. *Lynceus biformis* was roughly eight times more abundant in the egg bank than the other two species.

Figure 4 shows the egg density ($d_{b,s}$) distributions of these three species, and Figure 5 presents the gradients in Siangtian Pond. Eggs of *B. kugenumaensis* were mostly concentrated in three places (accounting for 43.7 % eggs throughout the 58 total sampling points; Fig. 5A), which are all small pits (diameters ranging from 0.5 to 1 m) and the deepest places in Siangtian Pond (i.e. three circles marked with $d_{b,s}$; Fig. 4A). *Eulimnadia braueriana* eggs clumped mainly along the less vegetated path (Fig. 4B) and inclined towards the western periphery of the path (Fig. 5B). *Lynceus biformis* eggs were distributed across a wider range (Fig. 4C), especially towards the southern common rush region and the pool periphery. Moreover, the *L. biformis* distribution was separated into northern and southern portions rather than being continuous throughout the basin (Fig. 5C).

There was no significant correlation between the log-transformed $d_{b,s}$ and distance from the morphometric center, D_s ($r^2 = 0.01$, 0, and 0, $p = 0.49$, 0.69, and 0.96 for *B. kugenumaensis*, *E. braueriana*, and *L. biformis*, respectively; Fig. 6). However, the correlation between the log-transformed $d_{b,s}$ and transformed distance (D_s') was significant in *B. kugenumaensis* ($r^2 = 0.39$, $p < 0.001$), but was insignificant in both *E. braueriana* ($r^2 = 0.01$, $p = 0.41$) and *L. biformis* ($r^2 = 0$, $p = 0.78$) (Fig. 7). For *B. kugenumaensis*, $d_{b,s}$ decreased linearly with the increase of D_s' (Fig. 7A). The $d_{b,s}$ of *E. braueriana* were more concentrated around D_s' at 25-30 m (Fig. 7B), but were more even across D_s' for *L. biformis* (Fig. 7C).

Table 2 summarizes the maturation time (*ts*) for this branchiopod community during 2006-2013. *Eulimnadia braueriana* was the first species to reach sexual maturity, with *ts* ranging from 7 to 9 *DAII* (n = 8), followed by *B. kugenumaensis* with a *ts* of 9-12 *DAII* (n = 14; including six successful recruiting hydroperiods in 2004 and 2005). *Lynceus biformis* was the last species to reach maturation at 13-16 *DAII* (n = 3).

Discussion

The egg bank is a key component for large branchiopods to persist in stochastic temporary pools, and egg bank size plays an important role in quantitatively evaluating the local population status. In addition, the egg bank spatial structure results from long-term population dynamics between recruitment and depletion (Brendonck and Riddoch, 2000; Brendonck et al., 2000; Brendonck and De Meester, 2003), and its horizontal distribution may demonstrate potentially important egg bank "hotspots" as the core hatching regions for the population. In other words, egg bank spatial structures are informative for understanding species composition and abundance, and establishing appropriate conservation actions (Ripley et al., 2004; Boven et al., 2008). Since Siangtian Pond is characterized by rapidly fluctuating water levels and swift changes in available microhabitats for inhabitants, sediment sampling from a single or a few places as has been done in small rocky pools (Brendonck and Riddoch, 2000; Vanschoenwinkel et al., 2010) may not be sufficient to reflect spatial structures in these larger habitat types. Similarly, traditional line transects (Thiéry, 1997; Mura, 2004, 2005) could miss some places due to uneven pool morphometry and biased horizontal distribution results. Thus, I systematically sampled a grid with more effort across the core inundation area, as suggested by Maffei et al. (2002) and Brendonck and De

Meester (2003), which provided a more accurate egg bank size estimation and finer spatial distribution resolution. Relatively small sampling areas of only 0.03 % in the core range could reduce disturbance to the egg bank as a whole, while maintaining the representation of my results. Although underestimation may still occur when eggs covered in sediment were not counted, the estimated egg bank size should be close to the functional egg bank size. My method can serve as a standard procedure for evaluating egg bank spatial structures in large temporary wetlands, and the results can be seen as a direct indicator of population status.

Results demonstrated that the egg bank size (E_b) of *B. kugenumaensis* was 2.1×10^8, which is on a similar order of magnitude as its recruiting egg number per cohort (2.5×10^7 to 1.2×10^8, estimated in Huang et al., 2011). Thus, I conclude that egg accumulation of *B. kugenumaensis* at this pond is quite limited, and the restriction is probably due to high egg depletion caused by factors such as abortive hatches, senescence, or mortality (Hairston et al., 1995; Brendonck and De Meester, 2003). On the other hand, reserving more eggs in the sediment than the amount necessary to ensure buffering capacity against environmental stochasticity may be wasteful since egg viability decreases over time (Marcus et al., 1994; Hairston et al., 1995; Mura, 2005). Therefore, unlimited egg accumulation may not be advantageous to population persistence, as it is better to risk eggs in hatching attempts than to allow them to expire over time.

Although this study showed a fixed value of E_b at a given time, it should fluctuate over time because populations will experience diverse abortive hatching and successful recruiting probabilities (Brendonck, 1996; Simovich and Hathaway, 1997; Brendonck et al., 2000). Previous studies demonstrated that fecundity varied across different cohorts (Huang et al., 2011) and was related to body length or growth patterns (Huang et al., 2010), and thus the abundance of E_b may be further biased. If any branchiopod species can persist in Siangtian Pond, long-term tradeoffs between the depletion and recruitment of eggs must be positively balanced. Comparing the mean egg density ($\overline{D_b}$) of *B. kugenumaensis*, estimated in this study as 29,514 (± 26,539) per m^2, with cohort fecundities of 10,631-36,425 eggs per m^2 in 2005 (Huang et al. 2011), I expect that $\overline{D_b}$ should be balanced around 30,000 eggs per m^2, which leads to an E_b around 2×10^8 for *B. kugenumaensis*. On the other hand, while *L. biformis* was the most abundant species in the egg bank, it is unclear if this numerical dominance relates to particular factors such as colonizing history, fecundity, low abortive hatching, or low egg mortality. Speculatively, *L. biformis* may have prevailed in recent longer and colder hydro-periods, thus accumulating a larger egg bank, although this would need more studies to verify.

Results further showed a significant variation in the horizontal distribution patterns of egg banks among the three species: *B. kugenumaensis* is mainly clumped in the three deepest places, *E. braueriana* is distributed along the less vegetated portions, and *L. biformis* is spread across a wider range towards the southern common rush region. Patchy egg spatial distribution has been demonstrated in other studies focusing on either multiple sympatric species (Carvalho and Wolf, 1989; Thiéry, 1997) or single species (Mura, 2004, 2005). The uneven and differentiated egg bank distribution implies that these three species may have diverse microhabitat preferences (Hamer and Appleton, 1991; Thiéry, 1997) or different egg dispersal mechanisms. If spatial usage of free-living populations is the only factor for constructing egg bank spatial structures, adults should display a fixed distribution pattern both in and throughout the hydroperiod. However, Wang et al. (2012) demonstrated that large branchiopods showed differentiated horizontal distributions in Siangtian Pond, but not in a consistent pattern throughout the hydroperiods in 2008. The rapidly fluctuating water level may have disturbed and imposed strong restrictions on microhabitat selection. Long-term monitoring on adult distributions across more hydroperiods should help clarify any patterns and their impact on egg bank spatial structures.

I found that egg density decreased for *B. kugenumaensis* as the transformed distance from the three deepest places (D_s') increased, but not for the other two species. Two simplified distribution patterns in which the eggs distributed peripherally (Hildrew, 1985; Thiéry, 1997; Mura, 1991, 2005) or centrally (Mura, 1991, 1997) have been proposed and can be attributed to active and passive strategies, respectively. Spawning around the pool periphery is hypothesized to be an adaptive strategy ensuring that dormant eggs will hatch when the hydroperiod is longest (Hildrew, 1985; Thiéry, 1997). This can be called an “active” strategy, achieved either by adults that actively lay eggs on the edge (Mura, 2005) or due to floating eggs blown to the margins by wind (Mura, 2004). Mura (1991) suggested that in the case that eggs are distributed in deeper regions, adults are restricted by the extent of inundation areas when they mature; they have no choice but to release the dormant eggs in the limited immersed region. A centrally distributed egg bank is thus more independent of adult microhabitat preferences, and would be a “passive” strategy. The *B. kugenumaensis* egg density, centralized in the three deepest portions of Siangtian Pond, fits my prediction for a late maturation species. This distribution pattern conforms to my hypothesis of the correlation between horizontal egg bank distribution and population maturation time. Although other factors may exist, spatial usage by the active population played a principal role and approximately determined the dormant egg hotspots. *Branchinella kugenumaensis* first spawns 9-12 days after inundation. As hydroperiods typically last from 11-17 days (Huang et al., 2010), matured *B. kugenumaensis* are usually limited to spawning in the three deepest places which still hold water at that time. In other words, *B. kugenumaensis* adopted the passive strategy in Siangtian Pond.

For the other two species, linear correlations between egg density and D_s' were both insignificant, implying that another mechanism was involved. *Eulimnadia braueriana* was predominately found around the 25-30 m range of D_s', especially near the less vegetated path in the northwest region. The eggs of *L. biformis* were relatively uniformly distributed through the entire range of D_s', particularly towards the southern part. The highest density of eggs for *E. braueriana* nearly approached the margin of the 3 m isobath core area. This characteristic of the egg bank for *E. braueriana* fits partially with my prediction for early spawning species. Since *E. braueriana* was the earliest one to mature at 7-9 days, it had more opportunity to adopt the active strategy to spawn more widely beyond the inundated area of the 2 m isobath. However, it should be noted that the spatial trend is towards the northwest region rather than even distribution throughout the periphery. Neither is the distribution towards the leeward southwest region during the prevailing northeast monsoons, which may indicate that factors other than the inundation area at maturation or wind action could affect the egg distribution. I speculate that *E. braueriana* may have a specific microhabitat preference for the less vegetated path, which crosses the basin of Siangtian Pond. Nevertheless, the uneven distribution throughout the whole path implies the potential effect of other factors, and should be clarified by more studies on the space utilization of free-living individuals and abiotic mechanisms.

Additionally, *L. biformis* was the last species to reach sexual maturity, requiring about two weeks to produce the first clutch. Therefore, its potential spawning area should be limited even more than what *B. kugenumaensis* experienced, and theoretically *L. biformis* could only adopt the passive strategy. However, the eggs of *L. biformis* were distributed more widely and peripherally towards the southern part beyond the common rush region, which is opposed to my prediction for late maturing species. The contradiction means that the inundation area at maturation cannot explain its horizontal distribution pattern. Other factors may redistribute and determine the egg bank spatial structure. Air dried eggs of *L. biformis*, for instance, become invaginated (Olesen et al., 2013), probably allowing them to trap air, remain floating, and thus be blown by seasonal winds more easily from the northeast monsoons to leeward (Thiéry, 1997; Brendonck and De Meester, 2003; Mura, 2004). Plants could also delay drifting of eggs, entangle them more often in the area with dense vegetation, and cause their distribution to be inclined towards the southern region of Siangtian Pond. Moreover, Pinceel et al. (2013) demonstrated that floating eggs had higher hatching fractions than sinking ones and that these two types of eggs could interchange. It thus follows that different proportions of drifting eggs among populations may construct species-specific egg bank spatial structures because of the varied hatching fractions and dispersal abilities between floating and sinking eggs (Pinceel et al., 2013). In other words, eggs of the three large branchiopods in Siangtian Pond could be set by multiple factors in a complex manner. While factors such as wind action and egg buoyancy could be important, the egg bank of *B. kugenumaensis* may have been affected more by maturation time than *E. braueriana* and *L. biformis*.

Species-specific patterns of egg bank distribution could drive divergent evolutionary adaptations in hatching phenology. For example, centrally distributed *B. kugenumaensis* eggs may evolve a number of mechanisms for responding positively to environmental cues that indicate suitable growth conditions, including fine control of osmotic pressure (Hall and MacDonald, 1975) at lower concentrations (Brown and Carpelan, 1971; Brendonck et al., 1998; Vanschoenwinkel et al., 2010) and light exposure (Vanhaecke et al., 1981; Mitchell, 1990; Murugan and Dumont, 1995; Kuller and Gasith, 1996), while other mechanisms are evolved for conservatively lowering hatching rates per hydroperiod to reduce the risk of abortive hatching. The more peripherally distributed *E. braueriana* and *L. biformis*, on the other hand, may be selected for higher hatching rates because their eggs would be immersed when precipitation is abundant enough to extend the water area towards the periphery, and thus are less sensitive to their aquatic surroundings. Further studies on factors affecting egg bank spatial structure and hatching phenology should help clarify the distribution mechanisms and hatching strategies of large branchiopods in Siangtian Pond.

To conclude, I estimated the egg bank sizes of three sympatric large branchiopods in Siangtian Pond. *Lynceus biformis* was the most abundant in the egg bank, while the functional egg bank size of *B. kugenumaensis* was on a similar magnitude to its cohort fecundity, indicating restricted egg accumulation in the egg bank. Results in different horizontal egg bank distributions among the species could be partially related to the duration of population maturation and hydroperiod. *Branchinella kugenumaensis* dominated the three deepest areas that should result from late spawning and a limited inundation area. *Eulimnadia braueriana* reproduced earliest and had eggs predominantly near the margins, but the uneven pattern may imply special microhabitat preferences. *Lynceus biformis* reproduced last but also distributed peripherally, indicating the influence of factors other than developmental schedule and water level fluctuation. Mechanisms such as egg floatation and wind action may also be influential and may serve as the basis for further hypotheses about constructing egg bank spatial distribution of large branchiopods in future studies.

Table 1. Mean egg density per m^2 ($\overline{D_b}$, with standard deviation) and the functional egg bank size (E_b) of *B. kugenumaensis* (Bk), *E. braueriana* (Eb), and *L. biformis* (Lb).

	Bk	Eb	Lb
$\overline{D_b}$	29,514 (± 26,539)	31,969 (± 30,626)	241,184 (± 72,918)
E_b	2.1×10^8	2.3×10^8	1.7×10^9

Table 2. Year, inundation episode, hydroperiod (*HP*; days), and maturation time (days after initial inundation, *DAII*) for *B. kugenumaensis* (Bk), *E. braueriana* (Eb), and *L. biformis* (Lb). NRM: not reached maturity; NH: not hatched; –: no record. Data of 2004 and 2005 are cited from Huang et al. (2010).

Year	**Episode**	***HP***	**Bk**	**Eb**	**Lb**
2004	P3	50	10	–	–
	P4	16	10	–	–
2005	P1	14	10	–	–
	P2	13	10	–	–
	P3	11	9	–	–
	P4	13	10	–	–
2006	P1	6	NRM	NRM	–
	P2	14	10	8	–
2007	P1	16	11	7	–
	P2	15	11	9	–
	P3	17	12	9	–
2008	P1	6	NRM	NRM	NH
	P2	32	9	7	16

2010	P1	23	NRM	NRM	NRM
2011	P1	5	NRM	NRM	NH
2012	P1	17	11	9	15
	P2	9	NRM	NRM	NRM
2013	P1	14	11	9	NRM
	P2	5	NRM	NRM	NH
	P3	24	10	9	13

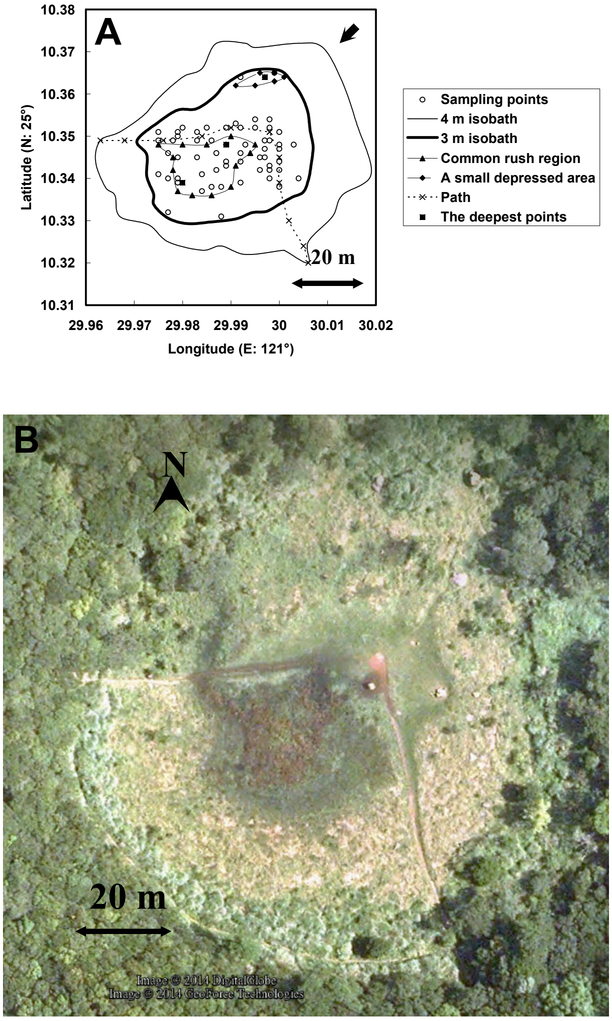
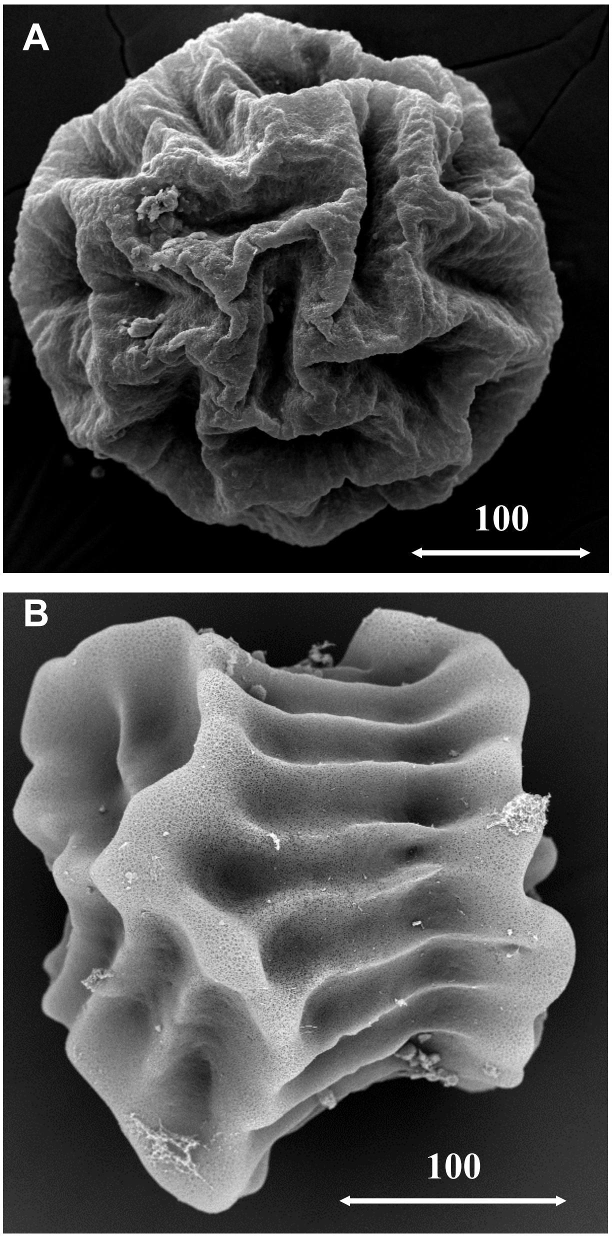


Figure 1. (A) Sediment sampling points in Siangtian Pond from the main inundated range of 3 m isobath (the bold solid line; with scale bar); the bold arrow at top right corner shows the dominant wind direction of northeast monsoons. (B) The airscape of Siangtian Pond (Source: 25°10’26’’ N, 121°29’ 56’’ E. Google Earth. Image date: 1 January 2006. Accessing date: 7 February 2014).


A
100
B
100

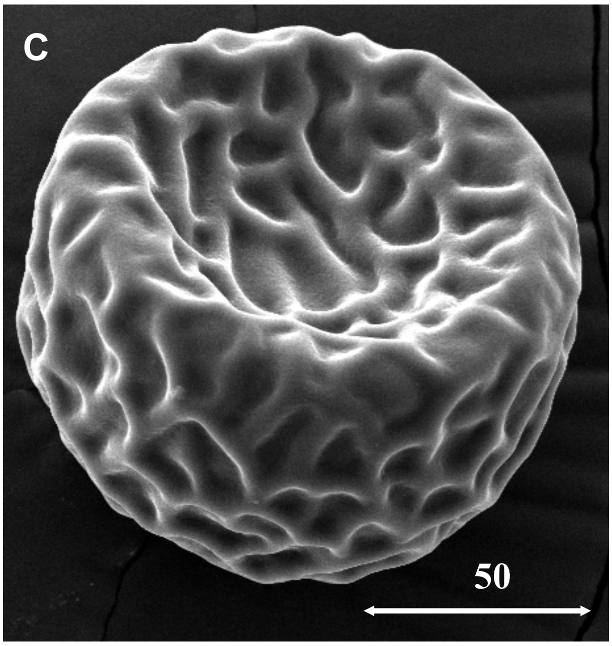


Figure 2. Eggs. (A) *B. kugenumaensis*; (B) *E. braueriana*; (C) *L. biformis* found in the sediment (with scale bar; the lengths are different).

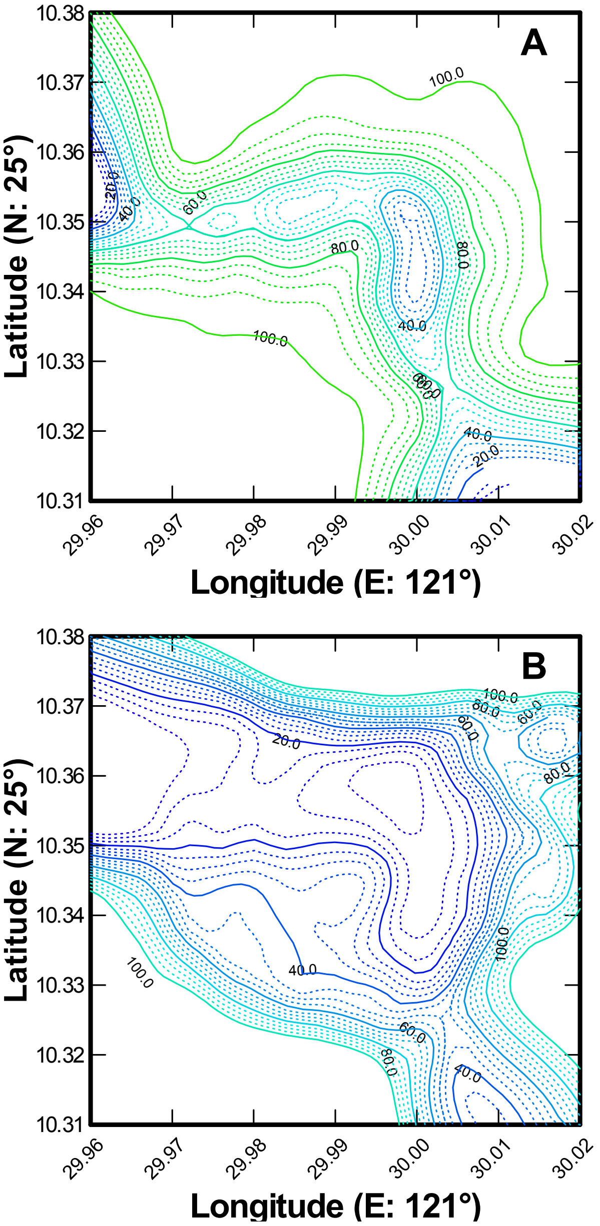


Figure 3. Vegetation characteristics of Siangtian Pond. (A) Vegetative coverage (C_s); (B) vegetation height (H_s). Each of the contour lines represents the isobath with the same value.

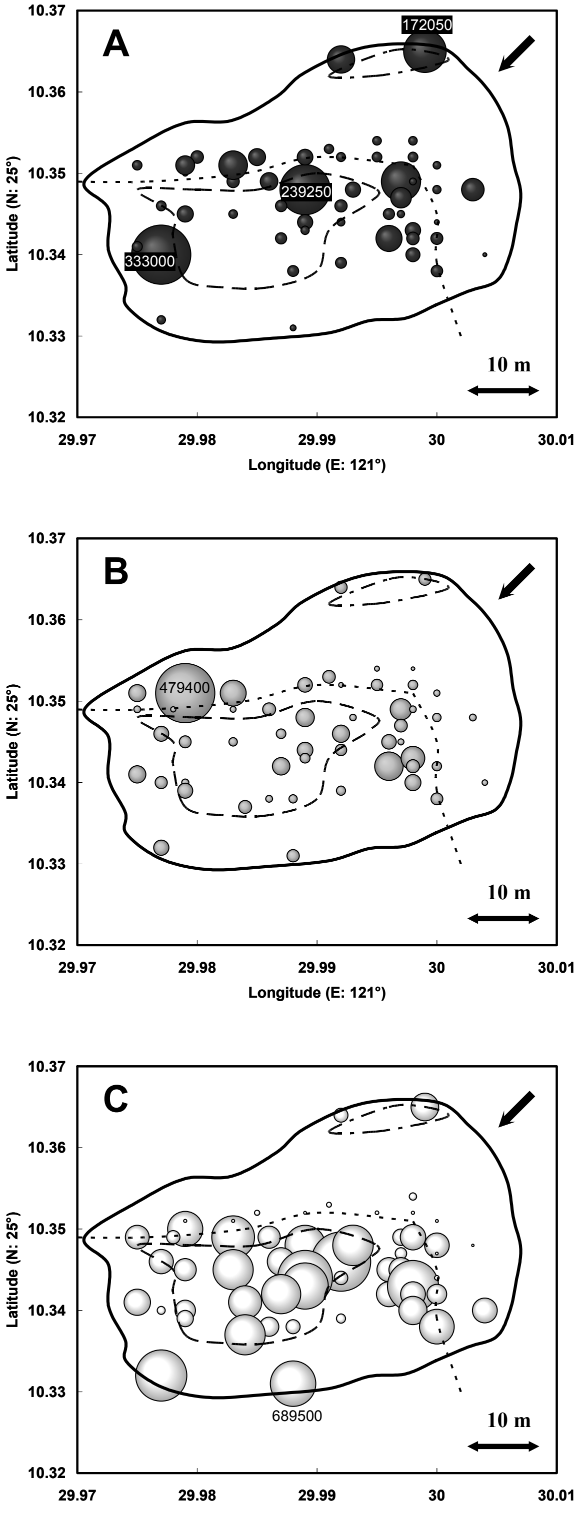
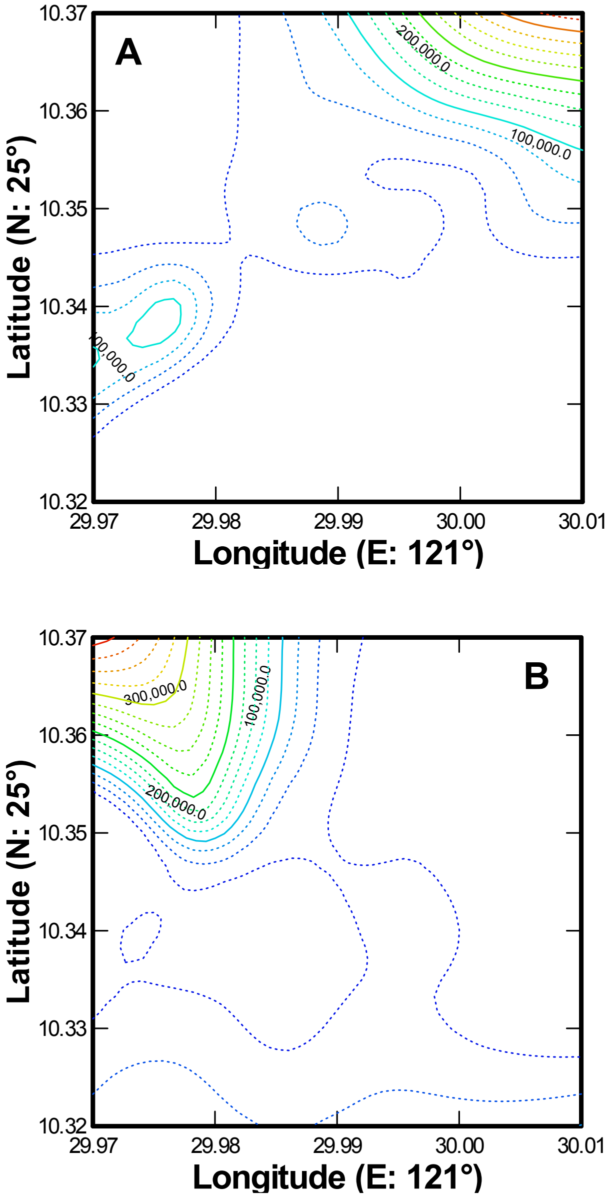


Figure 4. Egg density ($d_{b,s}$) distributions. (A) *B. kugenumaensis*; (B) *E. braueriana*; (C) *L. biformis*, within the 3 m isobath (with scale bar). The numbers represent the $d_{b,s}$ of

those sampling points for certain places. Common rush region, a small depressed area, and the path are all showed by dotted lines. The bold arrow at top right corner shows the dominant wind direction of northeast monsoons.


A
10.37
10.36
10.35
10.34
10.33
10.32
Latitude (N: 25°)
29.97
29.98
29.99
30.00
30.01
Longitude (E: 121°)
200,000.0
100,000.0
100,000.0
B
10.37
10.36
10.35
10.34
10.33
10.32
Latitude (N: 25°)
29.97
29.98
29.99
30.00
30.01
Longitude (E: 121°)
300,000.0
100,000.0
200,000.0

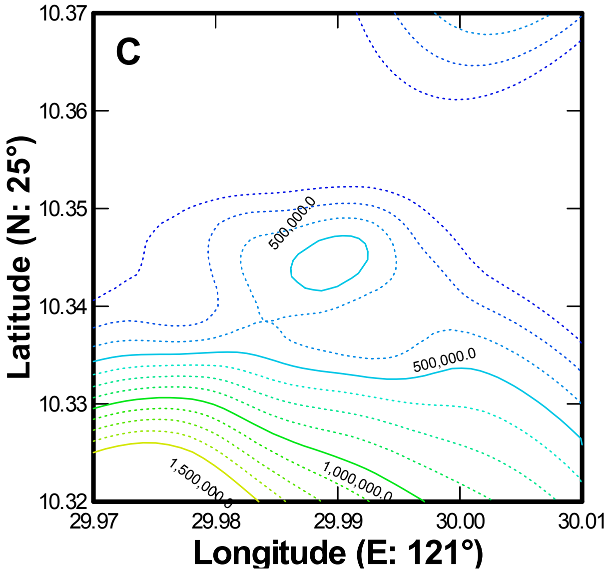


Figure 5. Egg density ($d_{b,s}$) gradients. (A) *B. kugenumaensis*; (B) *E. braueriana*; (C) *L. biformis*. Each of the contour lines represents the isobath with the same $d_{b,s}$.

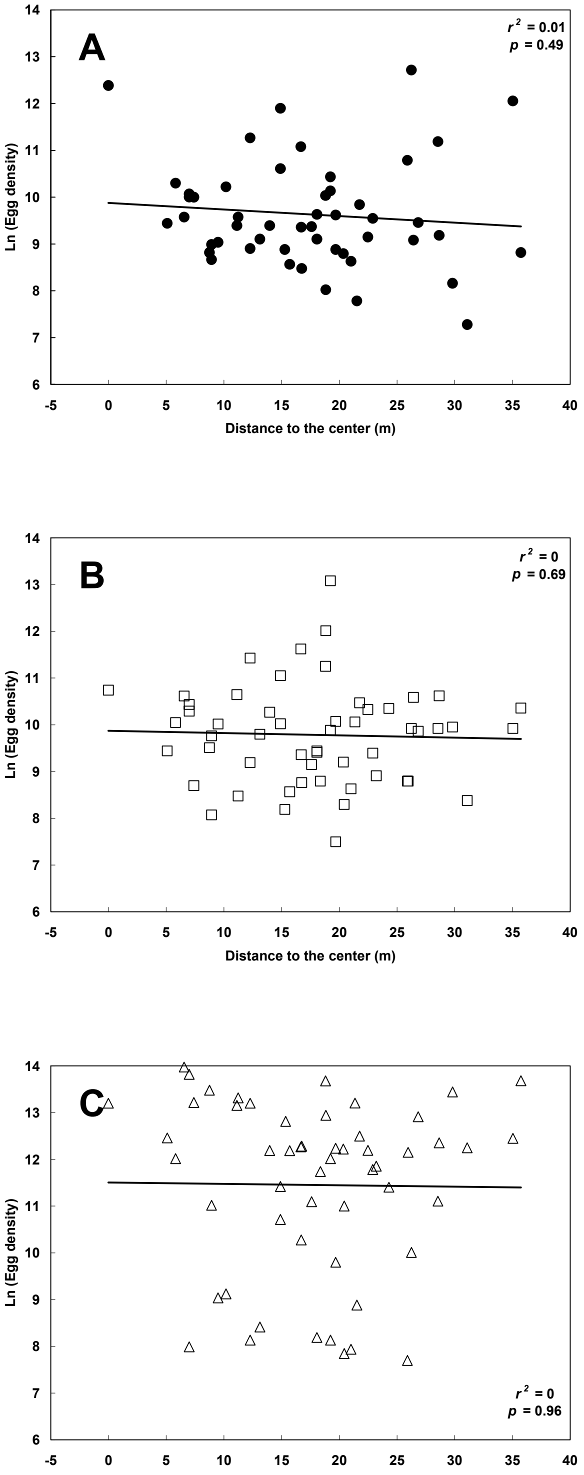


Figure 6. Linear regression between egg density ($d_{b,s}$) after log-transformation and distance to the center (D_s). (A) *B. kugenumaensis*; (B) *E. braueriana*; (C) *L. biformis*.

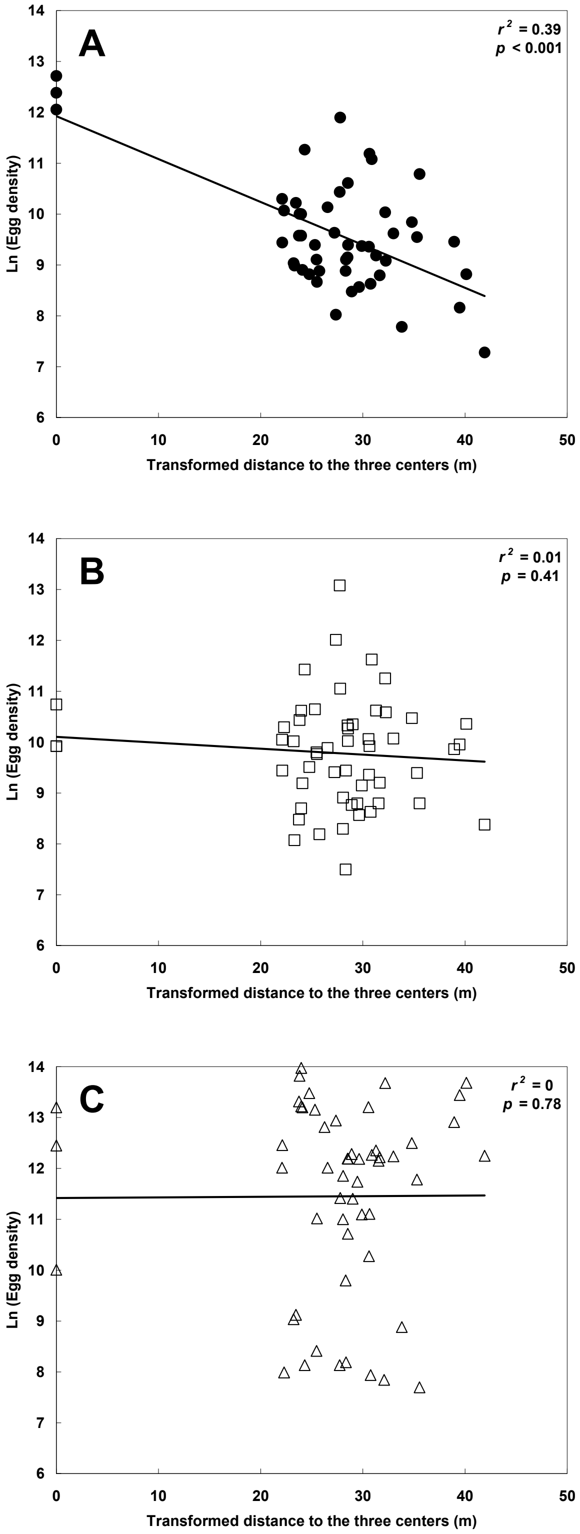


Figure 7. Linear regression between egg density ($d_{b,s}$) after log-transformation and the transformed distance to the three deepest places (D_s'). (A) *B. kugenumaensis*; (B) *E.*

braueriana; (C) *L. biformis*.

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Chapter 4: Terminating dormancy: hatching phenology of sympatric large branchiopods in Siangtian Pond, a temporary wetland in Taiwan

Abstract

Large branchiopods adapt to stochastic temporary wetlands by means of rapid development and production of dormant eggs. Unhatched eggs build the egg bank that bridges the frequent unfavorable periods. Dormancy termination is complex not only in appropriate hatching times, but also in hatching fractions. I investigated the parameters of hatching phenology including the initial hatching time, hatching duration, hatching rate, and cumulative hatching rate of two sympatric large branchiopods, *Branchinella kugenumaensis* (Ishikawa, 1895) and *Eulimnadia braueriana* Ishikawa, 1895, in Siangtian Pond in northern Taiwan, to explore the hatching strategies. In the laboratory studies, substrate samples with quantified egg numbers were inundated and dehydrated periodically and repetitively until no further hatching occurred, and the according parameters were recorded and calculated. I found that for both species, the hatching time was focused early in the hydroperiod. Most larvae hatched in the early hydroperiods. Overall, *E. braueriana* had a higher hatching rate through hydroperiods, and a higher cumulative hatching rate (82.9 %) than *B. kugenuaensis* (47.5 %). Early and concentrated hatching in a given hydroperiod could be adaptive, leading individuals to mature before the end of hydroperiod and reducing the frequency of abortive hatching. Since the hydroperiods of Siangtian Pond were synchronous with torrential rains, to incline the hatchings toward early hydroperiods is more preferable than to spread over several hydroperiods under the predictable hydroregime. Generally, the hatching phenology of *E. braueriana*, i.e., the longer hatching duration and higher hatching rate, means that it could be better adapted to the hydroregime of Siangtian Pond.

Keywords: Anostraca, *Branchinella kugenumaensis*, *Eulimnadia braueriana*, hatching, hydroperiod, hydroregime, Spinicaudata

Introduction

Temporary wetlands are dynamic habitats characterized by recurrent phase changes between wetland and upland (Williams, 1985; Schwartz and Jenkins, 2000). Depending on climate regime and geography, the water may come from various sources including melting snow (Daborn, 1975; Mossin, 1986; Mura, 2001), flooding (Hamer and Rayner, 1996; Eder et al., 1997; Merta, 2003), rainfall (Marcus and Weeks, 1997; Brendonck et al., 1998; Huang et al., 2010), and artificial irrigation (Grygier et al., 2002; Su and Mulla, 2002). However, hydroperiod timing and duration are typically unpredictable and unstable; thus these habitats are stochastic.

Large branchiopod crustaceans (Anostraca, Notostraca, Laevicaudata, Spinicaudata) are common representatives of temporary wetlands (Brendonck et al., 2008). They have evolved specific strategies to cope with environmental stochasticity. They can grow and reproduce rapidly during the favorable periods, and produce resistant dormant eggs to bridge the unfavorable periods (Hildrew, 1985; Weeks et al., 1997; Brendonck et al., 2000). Therefore, the life cycle of large branchiopods is composed of two distinct stages: the active stage in the water body, and the dormant stage in the substrate. These two stages interact closely through benthic-pelagic coupling (Brendonck and De Meester, 2003; Gyllström and Hansson, 2004) for population persistence in local habitats.

In this benthic-pelagic coupling, hatching from the dormant stage is usually asynchronous and spreads over several hydroperiods. This phenomenon preserves long term fitness in a tradeoff with decreasing current fitness and has been hypothesized as a bet hedging strategy (Philippi and Seger, 1989) to reduce the risk of abortive hatching (Brendonck, 1996; Brendonck et al., 1998), wherein a given cohort fails to mature and recruit eggs before the end of hydroperiod. The remaining unhatched eggs would accumulate in the substrate to become an egg bank for future hydroperiods (Brendonck and De Meester, 2003). Dormant eggs are a long-lived stage, acting as the key for large branchiopod viability and as a bridge through frequent catastrophes during unfavorable periods.

Dormant eggs need to be sustained in sufficient quantity and quality to maintain a given population. Initializing dormancy could be relatively simple. Since most branchiopods produce dormant propagules directly (Cáceres, 1997), the better strategy is to mature and reproduce as soon as possible before the hydroperiod ends (Hildrew, 1985; Brendonck et al., 2000). However, terminating dormancy is complex as regards detecting environmental signals as well as the pressure of synchronizing with the local long term hydroregime. Hatching should occur at the right time, not only when water is present, but also at the appropriate “time window” within the hydroperiod (Brendonck and De Meester, 2003; Jocqué et al., 2010; Rossi et al., 2013). Additionally, although hatching generally bet hedges into several hydroperiods, there should be more hatching during the longer hydroperiods (Cohen, 1966) for recruiting more eggs and maintaining egg bank viability. Therefore, the long term tradeoff between risk spreading and maximizing cohort fecundity will determine the adaptive hatching phenology in specific hydroregimes (Brendonck and De Meester, 2003).

Siangtian Pond is a temporary wetland in Taiwan. The hydroperiod depends on unpredictable climate events such as typhoons and stationary fronts (Huang et al, 2010). Previous studies (Huang et al., 2010; 2011) focused on the life histories of fairy shrimp *Branchinella kugenumaensis* (Ishikawa, 1895) and on egg bank spatial structure (Wang et al., 2014) of sympatric *B. kugenumaensis*, *Eulimnadia braueriana* Ishikawa, 1895, and *Lynceus biformis* (Ishikawa, 1895). However, hatching phenology has remained unexplored. With different life histories and frequent abortive hatchings (Wang et al., 2014), what is the hatching phenology of each species? In this study, I examined the hatching phenology of both *B. kugenumaensis* and *E. braueriana* with varied maturation rates under the Siangtian Pond hydroregime.

Hatching fraction has been shown to be positively correlated with probability of successful recruitment (Brendonck et al., 1998; Brendonck and Riddoch, 2001; Maffei et al., 2005). Because the water level of Siangtian Pond reduces at a stable rate, the hydroperiod usually ends within 2-3 weeks without replenishing rainfalls (Huang et al., 2010). Under this characteristic hydroregime, I assumed that the successful recruitment probability of a species depends on its maturation time. If a population can reproduce early, I predicted that it would have a higher successful recruitment probability and thus a higher hatching rate through the hydroperiods. In addition, for the early maturing species, late hatching individuals still have chance to reproduce before the hydroperiod ends. Therefore, it may not be strongly selected for a more synchronous hatching with the initialization of a given hydroperiod. Accordingly, I predicted that the early maturing population would show a later initial hatching time and a longer hatching duration. As a whole, for *E. braueriana*, the earliest species to reach sexual maturity (Wang et al., 2014), I predicted that it would display a higher hatching rate, a later initial hatching time, and a longer hatching duration than *B. kugenumaensis*.

Materials and Methods

Study site

Siangtian Pond (25°10’26’’ N, 121°29’ 56’’ E; 818 a. s. l.) is a temporary wetland in Yangmingshan National Park in northern Taiwan. The basin is inundated after the heavy rains from typhoons, stationary fronts, or northeast monsoons. Although the maximal water depth can be more than 4 m at inundation, the hydroperiod usually lasts only 2-3 weeks. The rapid water reduction rate of 32 cm per day is due to the highly porous substrate. Occasionally, replenishing rains may prolong the hydroperiod longer than a month (Huang et al., 2010; Wang et al., 2012).

Laboratory hatching experiments

Superficial, dry substrate aliquots of 20 cm × 20 cm × 5 cm (length × width × depth) were collected from 58 sampling sites across the pond from 17 to 19 May 2010, and were kept dry in sealed zip type transparent plastic bags at room temperature (25 ℃) in complete darkness. Eggs were identified, quantified, and the density and egg bank size were estimated for each species (Wang et al., 2014).

I randomly selected four substrate samples from the original 58 samples, with 12 subsamples for each sample. Each subsample composed of 5 g randomly sampled substrate. Since eggs separated from the substrate may have different hatching phenology from those in substrate (Vandekerkhove et al., 2004), I did not isolate the eggs from the substrate before the hatching experiments. The estimated total number of branchiopod eggs per species was determined following the quantified methods in Wang et al. (2014).

Each subsample was immersed in 450 ml distilled water, maintained at 20-22 ℃ as is the prevailing temperature in the field during the natural hydroperiod (Wang et al., 2012), and cultured under a warm white lamp (Philips Helix HPF ES E27, 27W, 2700K, artificial spectrum, Ra = 81) for 24 hours to try to maximize the hatching performance (Vanhaecke et al., 1981; Mitchell, 1990; Murugan and Dumont, 1995; Brendonck, 1996; Horiguchi et al., 2009; Pinceel et al., 2013). Newly hatched nauplii of each species were identified and counted daily for a week (n_i, i: the i^{th} hydroperiod). Naupliar larvae can be readily distinguished, as *B. kugenumaensis* is larger (1 mm), more slender, and moves slower than *E. braueriana* (0.5 mm). Nauplii were removed to other tanks by plastic pipette for life history studies.

After a week of inundation, the substrate was air dried under the same temperature (20-22 ℃) for another week under complete darkness until dry and then reinundated with distilled water. In this repetitive inundation-dehydration cycle, hydroperiod was continued until no more hatching was observed after two rehydroperiods. I conducted a total of 262 inundation-dehydration replicates on 48 separate subsamples from May 2011 to April 2012. Since I aimed at hatching phenology, I neither measured the water parameters such as conductivity which has been demonstrated to be a good indicator of hatching occurrence (Brendonck et al., 2000; Vanschoenwinkel et al., 2010), nor monitored the chemical concentrations including glycerol and glycogen inside the eggs (Hall and MacDonald, 1975; Lavens and Sorgeloos, 1987; Murugan and Dumont, 1995), at the same time of experiment.

Hatching phenology

Four hatching phenology parameters included: initial hatching time ($T_{0,i}$; DAIH, days after initial hydroperiod), hatching duration ($T_{d,i}$; days), hatching rate (H_i; %), and cumulative hatching rate (CH_i; %). The definitions are summarized in Table 1. The H_i was calculated by

$$H_i = \frac{n_i}{N} \quad (1),$$

wherein N is the estimated total egg number of a given species in a given sample (quantified in Wang et al., 2014). The CH_i was calculated by

$$CH_i = \sum_{i=1}^{x} H_i \quad (2),$$

in which x represents the last hydroperiod with nauplii.

Hydroregime

To define the hydroregime of Siangtian Pond, I used the eleven year precipitation data from the Anbu Weather Station of the Central Weather Bureau from 2004 through 2014. I filtered the torrential rain frequencies (MATLAB, R2010a), which were defined as more than 200 mm precipitation per day (following the definition of the Central Weather Bureau), and accumulated monthly. I also summarized the monthly hydroperiod frequencies, wherein at least one large branchiopod species appeared before the pool dried out. The monthly frequencies of successful recruitment hydroperiods of the long term studied *B. kugenumaensis* were also calculated. It should be noted that were no collections in 2009 (Wang et al., 2014).

Data analysis

The $T_{0,i}$, $T_{d,i}$, and H_i were square root transformed for normalization before analysis because of the potential allometric effect. The differences of $T_{0,i}$, $T_{d,i}$, and H_i through hydroperiods were tested using general linear model (GLM) statistical analyses. Pairwise comparisons were made post hoc by the Games-Howell test due to unequal variances or sample sizes. Tendency of CH_i through hydroperiods was also explored by GLM. Differences of $T_{0,i}$, $T_{d,i}$, and H_i between species were analyzed by Kruskal-Wallis test. Because of small sample sizes, differences between frequencies of torrential rains and hydroperiods, and between hydroperiods and successful recruitment hydroperiods of *B. kugenumaensis* were using a two sample Kolmogorov-Smirnov test. All the analyses were performed by SYSTAT 12.

Results

The initial hatching time ($T_{0,i}$) was significantly different through hydroperiods in both *B. kugenumaensis* (GLM $F = 12.2$, $p = 0.001$) and *E. braueriana* (GLM $F = 24.8$, $p < 0.001$). For both species, $T_{0,i}$ was significantly later in the first hydroperiod (averaged 4.5 ± 1.8 and 4.9 ± 1.5 DAIH (days after initial hydroperiod) for *B. kugenumaensis* and *E. braueriana*, respectively) than the following hydroperiods (Games-Howell, $p < 0.001$), which were not significantly different between each pairs of hydroperiods (Games-Howell, $p > 0.05$; Fig. 1). However, the difference of $T_{0,i}$ was insignificant between *B. kugenumaensis* and *E. braueriana* (Kruskal-Wallis $K = 0.43$, $p = 0.51$), which averaged 2.7 ± 1.5 and 2.6 ± 1.5 DAIH, respectively.

The hatching duration ($T_{d,i}$) showed significant difference through hydroperiods in both *B. kugenumaensis* (GLM $F = 31.3$, $p < 0.001$) and *E. braueriana* (GLM $F = 22.3$, $p < 0.001$), but with different trends. For *B. kugenumaensis*, $T_{d,i}$ was not significantly different between the first and second hydroperiod (averaged 3.1 ± 1.8 and 3.0 ± 1.6 days for the first and second hydroperiod, respectively; Games-Howell, $p = 1$), but it was significantly different between the first two hydroperiods and the following hydroperiods (Games-Howell, $p < 0.001$; Fig. 2A). For *E. braueriana*, however, $T_{d,i}$ was significantly longer in the second hydroperiod (averaged 4.5 ± 1.5 days) than the other hydroperiods (Games-Howell, $p < 0.001$), which were not significantly different between each pairs of hydroperiods (Games-Howell, $p > 0.05$; Fig. 2B). Additionally, the difference of $T_{d,i}$ was significant between *B. kugenumaensis* and *E. braueriana* (Kruskal-Wallis $K = 15.2$, $p < 0.001$), wherein *E. braueriana* (averaged 3.1 ± 1.8 days) continued to hatch one day longer than *B. kugenumaensis* (averaged 2.3 ± 1.6 days). The hatching rate (H_i) was significantly different through hydroperiods in both *B. kugenumaensis* (GLM $F = 24.3$, $p < 0.001$) and *E. braueriana* (GLM $F = 86.5$, $p < 0.001$). For *B. kugenumaensis*, H_i was not significantly different between the first and second hydroperiod (averaged 14.8 ± 15.3 and 16.3 ± 11.9 % for the first and second hydroperiod, respectively; Games-Howell, $p = 0.72$). However, these two H_i were significantly higher than the following hydroperiods (wherein the H_i were only about 2 %; Games-Howell, $p < 0.001$; Fig. 3A). *Eulimnadia braueriana* demonstrated a different trend, first peaking at the second hydroperiod (averaged 32.0 ± 15.1 %), and second at both the first and third hydroperiods (averaged 15.6 ± 17.6 and 11.3 ± 7.9 % for the first and third hydroperiod, respectively). These three H_i were significantly higher than those of other hydroperiods (Games-Howell, $p < 0.001$; Fig. 3B). Generally, H_i was higher and inclined towards the first two to three hydroperiods for both species (Fig. 3). Moreover, H_i differed significantly between *B. kugenumaensis* and *E. braueriana* (Kruskal-Wallis $K = 5.7$, $p = 0.02$), wherein *E. braueriana* (averaged 13.8 ±

15.7 % per hydroperiod) was higher than *B. kugenumaensis* (averaged 10.0 ± 12.2 % per hydroperiod).

The cumulative hatching rate (CH_i) increased logarithmically through hydroperiods in both *B. kugenumaensis* (GLM $r^2 = 0.38$, $p < 0.001$; Fig. 4A) and *E. braueriana* (GLM $r^2 = 0.60$, $p < 0.001$; Fig. 4B). The CH_i reached a plateau in the fourth hydroperiod in *B. kugenumaensis* (accounting for 90.8 % of the total hatching fraction), but peaked later in the sixth hydroperiod in *E. braueriana* (accounting for 95.9 % of the total hatching fraction). In general, CH_i was higher in *E. braueriana* (82.9 %) than *B. kugenumaensis* (47.5 %).

Figure 5 shows the frequency of torrential rains, hydroperiods, and the successful recruitment hydroperiods of *B. kugenumaensis* in Siangtian Pond during 2004-2014. On average, there were 2.1 ± 1.0 hydroperiods per year. The torrential rains occurred from May to November, with the highest frequency during August-October ($n = 28$), which accounts for 77.8 % of torrential rains ($n = 36$). The difference between frequencies of torrential rains and hydroperiods was not significant (two sample Kolmogorov-Smirnov $D = 0.43$, $p = 0.52$). The peak frequency of hydroperiods occurred from July-October ($n = 16$) and was highly synchronous with torrential rains, accounting for 76.2 % of hydroperiods ($n = 21$). Additionally, the difference between hydroperiods and successful recruitment hydroperiods of *B. kugenumaensis* was not significant (two sample Kolmogorov-Smirnov $D = 0.29$, $p = 0.92$). Because four abortive hatchings occurred in June and July, most successful recruitment hydroperiods were concentrated in August-October ($n = 10$) for the long term *B. kugenumaensis* studies (accounting for 66.7 % of all successful recruitment hydroperiods; $n = 15$).

To summarize, my results showed that (1) $T_{0,i}$ was significantly longer in the first hydroperiod, but was not different between species, (2) $T_{d,i}$ lasted significantly longer during the first and second hydroperiods, and was even longer in *E. braueriana*, (3) H_i was higher from the first through the third hydroperiods, and was generally higher in *E. braueriana*, (4) CH_i increased rapidly for both species, and was higher by almost two fold in *E. braueriana*, and (5) the hydroperiods were highly synchronous with the occurrence of torrential rains.

Discussion

Large branchiopods rely on dormant eggs and the egg bank to bridge unfavorable periods (Brendonck and De Meester, 2003). Although dormant eggs can resist extreme environments and remain viable for a certain time (Fryer, 1996), the egg bank could be depleted by abortive hatching, mortality, and predation (Brendonck and De Meester, 2003; Waterkeyn et al., 2011). For long term, local population persistence, residents must recruit eggs to maintain the egg bank (Bishop, 1967a). Embryos have to terminate dormancy, hatch, and grow to maturity so they can reproduce. Facing environmental stochasticity, however, each hatching event is a bet. Under a given hydroregime, an endemic population would evolve a specific hatching phenology (Brendonck, 1996) to reduce the risk of abortive hatching. Therefore, studying the hatching phenology helps us to clarify the adaptive strategy in local habitats, and the comparison between sympatric species can even reveal how the hydroregime affects hatching phenologies of species with different life histories. Hatching phenology can be combined with demographic parameters of the active stage to model benthic-pelagic coupling dynamics, and develop proper conservation management strategies.

Since hatching is usually highly varied (Prophet, 1963; Scott and Grigarick, 1979; Hildrew, 1985; Brendonck et al., 1996; Mura and Zarattini, 1999; Mura, 2004), I applied repetitive hydroperiods in laboratory cultures to explore general hatching patterns of a local population. Although my indoor culturing method might not provide exact comparative condition to the field, it supplies a feasible way to obtain detailed parameters that could be easily missed during the beginning of a hydroperiod due to dangerous torrential rains. Moreover, the infrequent hydroperiods and naupliar larvae in Siangtian Pond may not be able to offer enough sample sizes for demonstrating reliable hatching phenologies even after ten years. Accordingly, although the results of indoor experiments should be explained carefully (Cáceres and Schwalbach, 2001), I suggest using substantial laboratory cultures for more efficiently investigating the hatching phenology of freshwater zooplankton which have a distinct dormant stage in their life cycles.

Several parameters of hatching phenology are under selection and can be used to reveal hatching strategies. First of all, time for initial hatching and hatching duration are critical. My results showed that for both *B. kugenumaensis* and *E. braueriana*, the initial hatching time was early at 2-3 days after initial hydroperiod, and the hatching duration only persisted for about 3 days. Although Pinceel et al. (2013) suggested that delaying initial hatching time can help the population to grow under more abundant resources, such as pelagic algae and vegetation litter, this did not occur in our case. Moreover, it was proposed that short hatching duration may cause more serious intraspecific competition for food by synchronizing development (Pinceel et al., 2013). However, my results showed brief hatching duration. As other studies indicated, this kind of early and concentrated hatchings occurring within a few days after inundation or substantial environmental changes (Moore, 1959; Bishop, 1967a; Brown and Carpelan, 1971; Bernice, 1972; Khalaf and Hall, 1975; Hildrew, 1985; Mitchell, 1990; Kuller and Gasith, 1996; Brendonck et al., 2000; Su and Mulla, 2002; Saengphan et al., 2005; De Roeck et al., 2010) rather than spreading across longer duration could be a general hatching strategy of large branchiopods in temporary wetlands (Brendonck, 1996; Vanschoenwinkel et al., 2010). Because the hydroperiod is unstable, to hatch earlier could benefit the population by allowing sufficient time for reproduction before the hydroperiod ends unpredictably (Rossi et al., 2013). This could explain why the initial hatching time of *E. braueriana* did not differ significantly from *B. kugenumaensis*, which contradicts my prediction on initial hatching time of early maturing species.

Nevertheless, I found that *E. braueriana* continued to hatch for longer time, fitting my prediction. It matures 1-4 days earlier than *B. kugenumaensis* (Wang et al., 2014), so late hatched individuals still have chance to mature. In addition, both species showed significantly delayed initial hatching time in the first hydroperiod. This could mean that hatching is postponed by the previous unfavorable period (Mattox and Velardo, 1950; Hall, 1959). Eggs may need more time to complete embryonic development (Brendonck, 1996; Saengphan et al., 2005), activate diapause, and stimulate quiescence (Bishop, 1967b; Brendonck, 1996; Brendonck and De Meester, 2003) during the first hydroperiod after deposition, and be ready to hatch earlier in the following hydroperiods. On the other hand, environmental parameters of Siangtian Pond, especially the conductivity, should be monitored in the future for clarifying if hatching happens within a specific range.

The hatching fraction distribution is another important issue. I found that the hatching rate inclined towards the early 2-3 hydroperiods in both *B. kugenumaensis* and *E. braueriana*, and the cumulative hatching rate increased rapidly through hydroperiods. The hatching fraction was focused in early hydroperiods rather than spread into more hydroperiods by prolonged dormancy. Extending dormancy, which seemed not the case in these two species, has been predicted to happen if the environment and reproductive success are highly varied (Ripley et al., 2004; Maffei et al., 2005). This implies that the Siangtian Pond hydroregime could be predictable and stable for residential large branchiopods. Since the hydroperiods were synchronous with torrential rains, factors correlating with abundant water, such as low conductivity (Brendonck et al., 2000; Vanschoenwinkel et al., 2010; Rossi et al., 2013), could provide a reliable stimulus for hatching. Under a less stochastic hydroregime, it would be better to shift hatching towards early hydroperiods than to spread it over more hydroperiods (Ripley et al., 2004). Although bet hedging is a well known strategy of large branchiopods in temporary wetlands (Brendonck, 1996; Brendonck and De Meester, 2003), I suggest that the pattern would be shaped by environmental stochasticity (representing as successful recruitment probability and its variation) and vary among populations living in different hydroregimes. Future studies should do meta-analysis on hatching phenologies of populations and species across habitats for revealing if large branchiopods adopt specific hatching strategies in characteristic hydroregimes.

The hatching rate of *E. braueriana* was higher than *B. kugenumaensis* and fitted my prediction. Although the successful recruitment probability was the highest in *B. kugenumaensis* (Wang et al., 2014: Table 2), it is probably due to the shorter study period for *E. braueriana*, resulting in uneven population maturation time records between these species. *Eulimnadia braueriana* took 7-9 days to reach maturity, which is 1-4 days earlier than *B. kugenumaensis* (Wang et al., 2014). Since all of the hydroperiods from 2004 to 2005 sustained sufficiently for *B. kugenumaensis* to reproduce (Huang et al., 2010), it is reasonable to infer that *E. braueriana* would also reproduce successfully during these periods. Therefore, the successful recruitment probability of *E. braueriana* should be at least the same or even higher than *B. kugenumaensis*. Theoretically, a species would increase its hatching fraction if successful recruitment probability is high (Cohen, 1966) or less variable (Cohen, 1968;

Venable, 2007). The abortive hatching of *E. braueriana* could be less frequent, so its hatching rate needs not be as conservative as late maturing *B. kugenumaensis*. It is also possible that hatching mechanisms, i.e., osmotic pressure accumulation (Hall and MacDonald, 1975; Lavens and Sorgeloos, 1987), function differently between these two species. Eggs of *E. braueriana* could be more efficient in building sufficient gradients, or it might have a lower threshold of osmotic pressure for breaking the envelopes than *B. kugenumaensis*. The hatching mechanism merits further studies. Accompanying with initial hatching time and hatching duration, both *B. kugenumaensis* and *E. braueriana* invested more hatching in early hydroperiods, especially in the second one with earlier, longer, and more abundant hatching. Siangtian Pond averaged two hydroperiods per year, with the majority from August to October. The hatching phenologies of both species could help them to synchronize with favorable periods for recruiting more eggs. Additionally, the main hatching fraction of *E. braueriana* separated into three hydroperiods, one hydroperiod more than *B. kugenumaensis*. This means that *E. braueriana* has better buffering capacity against abortive hatchings.

In general, *E. braueriana* showed a longer hatching duration, a higher hatching rate across more hydroperiods, and a higher cumulative hatching rate than those of *B. kugenumaensis*. I suggest that the hatching phenology of *E. braueriana* could be better adapted to the specific hydroregime of Siangtian Pond. The higher hatching fraction should lead *E. braueriana* to recruit more eggs in the predictable hydroperiod after torrential rains. However, Wang et al. (2014) studied the egg bank spatial structure of this large branchiopod community and demonstrated that the egg bank size of *E. braueriana* was of a similar numerical magnitude as *B. kugenumaensis*. This indicates that *B. kugenumaensis* might adopt other strategies to cope with the inferior hatching performance. To maintain the egg bank size under poor hatchings, *B. kugenumaensis* might have higher survivorship before maturation or be more fecund. It is also possible that environmental factors eliminate more *E. braueriana* eggs from the egg bank. More studies on parameters of life history and egg mortality can help to explain the contradiction between hatching phenology and egg bank size, and to model the egg bank dynamics of these two species in Siangtian Pond.

To conclude, I found that the hatching time of both *B. kugenumaensis* and *E. braueriana* focused early in the hydroperiod. It implies that hatching earlier could be an adaptive strategy for residents of temporary wetlands, advancing the population maturation time before the unpredictable end of the hydroperiod and reducing the risk of abortive hatching. Additionally, the occurrence of naupliar larvae of both species inclined towards early hydroperiods rather than dispersed over several hydroperiods. The hydroperiods of Siangtian Pond were highly synchronous with torrential rains. Under the predictable hydroregime, it is more preferable to hatch when the environment is favorable than to extend dormancy. Generally, I demonstrated that *E. braueriana* had a longer hatching duration and a higher hatching fraction than *B. kugenumaensis*, indicating that the hatching phenology of *E. braueriana* could be better adapted to the Siangtian Pond hydroregime.

Table 1. Parameters of hatching phenology and the definitions. DAIH: days after initial hydroperiod.

Parameter	Abbreviation	Definition	Unit
Initial hatching time	$T_{0,i}$	The first day with newly hatched nauplii in the i^{th} hydroperiod	DAIH
Hatching duration	$T_{d,i}$	The time period from the first day till the last day with newly hatched nauplii in the i^{th} hydroperiod	Days
Hatching rate	H_i	The ratio of hatched nauplii divided by the total number of eggs in the substrate in the i^{th} hydroperiod	%
Cumulative hatching rate	CH_i	The ratio of cumulative hatched nauplii till the i^{th} hydroperiod divided by the total number of eggs in the substrate	%

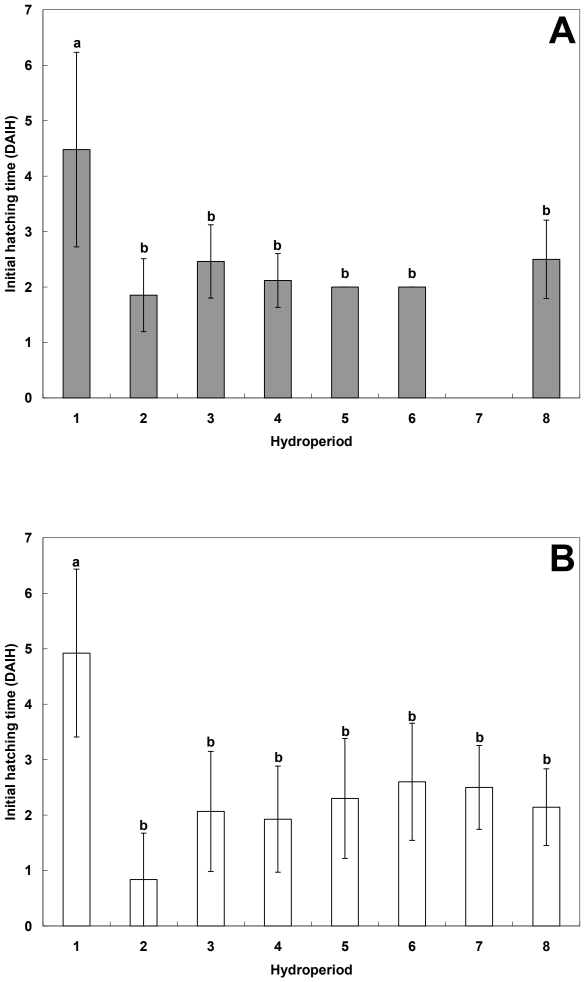

Figure 1. Trend of initial hatching time ($T_{0,i}$) through hydroperiods in (A) *B. kugenumaensis*, and (B) *E. braueriana*. The lowercase letters represent the groups after post hoc pairwise comparing, which were significantly different between groups (Games-Howell, $p < 0.001$), but were insignificant within a group (Games-Howell, $p > 0.05$).

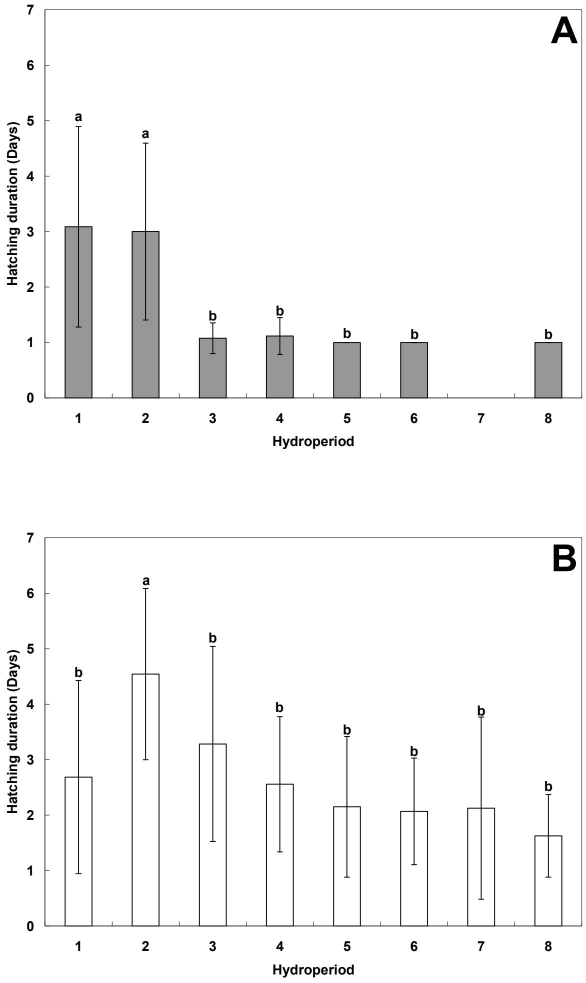

Figure 2. Trend of hatching duration ($T_{d,i}$) through hydroperiods in (A) *B. kugenumaensis*, and (B) *E. braueriana*. The lowercase letters represent the groups after post hoc pairwise comparing, which were significantly different between groups (Games-Howell, $p < 0.001$), but were insignificant within a group (Games-Howell, $p > 0.05$).

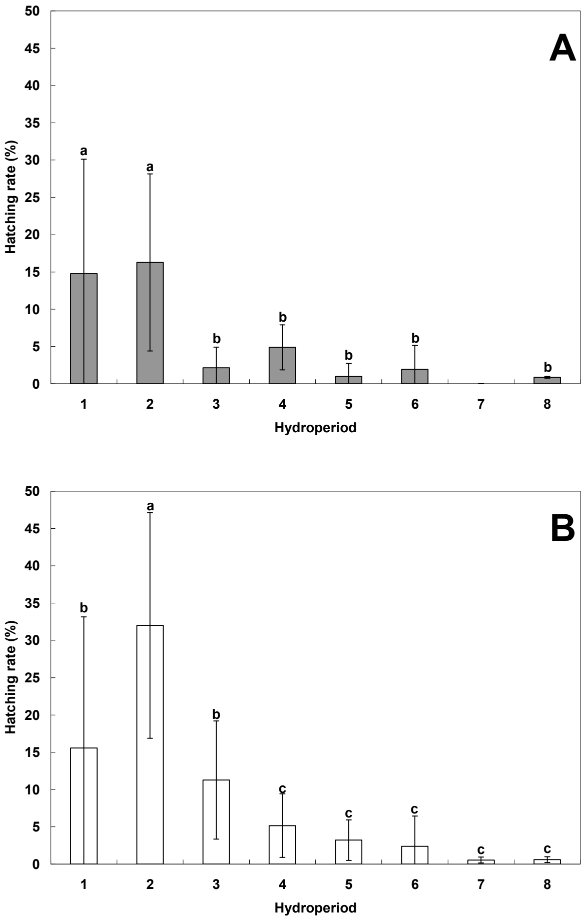

Figure 3. Trend of hatching rate (H_i) through hydroperiods in (A) *B. kugenumaensis*, and (B) *E. braueriana*. The lowercase letters represent the groups after post hoc pairwise comparing, which were significantly different between groups (Games-Howell, $p < 0.01$), but were insignificant within a group (Games-Howell, $p > 0.05$).

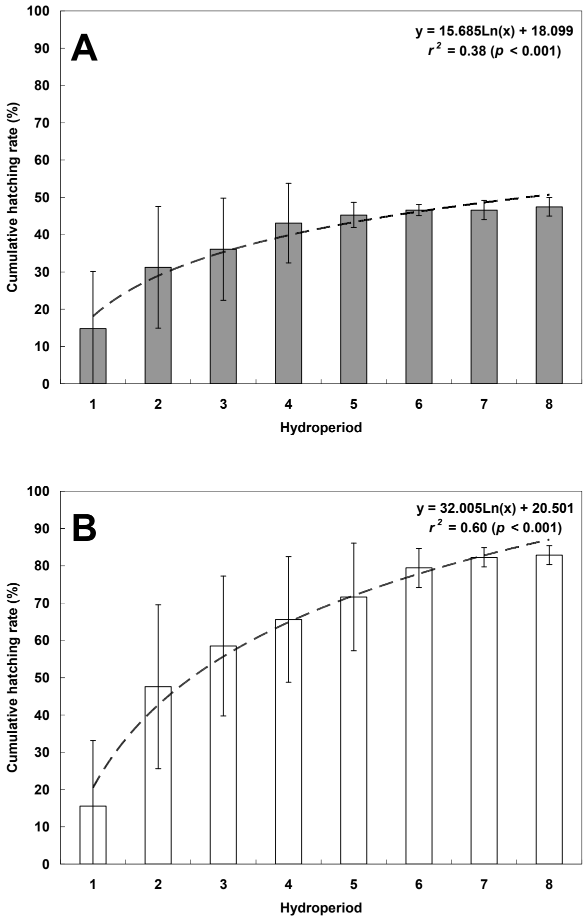

Figure 4. Cumulative hatching rate (CH_i) through hydroperiods in (A) *B. kugenumaensis*, and (B) *E. braueriana*. Both species increase logarithmically.

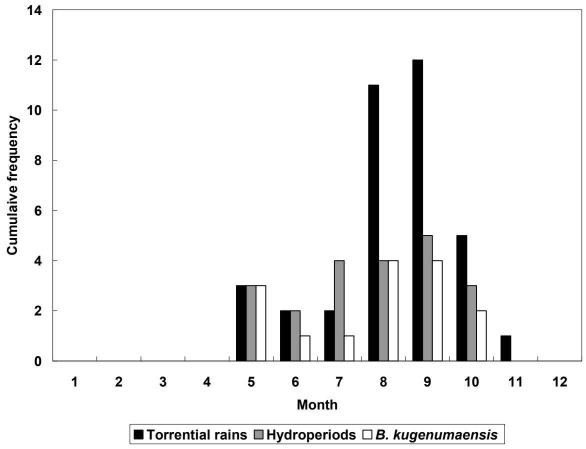

Figure 5. Cumulative frequency of torrential rains, hydroperiods, and successful recruitment hydroperiods of *B. kugenumaensis* in Siangtian Pond during 2004-2014.

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Chapter 5: Bet hedging in stochastic habitat: exploring the hatching strategy of sympatric large branchiopods in Siangtian Pond, a temporary pool in Taiwan

Abstract

Living in temporary pools, large branchiopods rely on resistant dormant eggs and egg banks to bridge through unfavorable periods. To reduce the depletion from abortive hatching, asynchronous hatching of eggs across several hydroperiods acts as bet-hedging strategy, avoiding local extinction. Hatching fraction in each hydroperiod plays the key role in maintaining the egg bank size, and the optimal hatching rate is predicted to be equal to the successful reproduction rate. I tested the difference between hatching rate and successful reproduction rate of *Branchinella kugenumaensis* and *Eulimnadia braueriana* in Siangtian Pond by data of indoor hatching experiments, field survey and hydroregime reconstruction from 67 years climatic records. Comparisons were performed under two scenarios, each of which means that a population hatches according to a hydroperiod or a year (with several hydroperiods), respectively. The latter scenario has a smaller range of fitness variation. Results showed that heavy rains served as a good predictor of hydroperiod. For *E. braueriana*, hatching rate only fitted to successful reproduction rate under the second scenario, but the consistency did not happen in both scenarios for *B. kugenumaensis*. I suggest that *E. braueriana* could adopt a more conservative hatching strategy, lowering the variation of fitness while at the cost of maximizing current fitness. On the other hand, the low hatching rate of *B. kugenumaensis* may result from no selection for optimization or physiological limitation, and this species may adopt other strategies to balance the inferiority in hatching performance.

Key words: *Branchinella kugenumaensis*, dormant egg, egg bank, *Eulimnadia braueriana*, hydroperiod, hydroregime

Introduction

Aquatic environments of temporary pools can be quite dynamic. The pool fluctuates alternatively between favorable and unfavorable periods (Williams, 1985; Schwartz and Jenkins, 2000). During favorable time, the basin contains water. However, conditions usually turn to be catastrophic during unfavorable periods, in which the water may totally dry out (Huang et al., 2010) or under extreme temperature, even completely freezing (Mossin, 1986). Depending on the climate regime and geology, hydroregime may differ significantly across habitats (Jocqué et al., 2010). Generally, the inundation is unpredictable and the hydroperiod is unstable, as in the representative freshwater rock pools (Brendonck et al., 2010).

Facing the stochastic changes, organisms inhabiting in temporary pools usually take two kinds of adaptive strategies, either emigrating out to other places (e.g., insects; Group 4 in Wiggin et al., 1980), or entering the resistant stage when environment becomes unfavorable (e.g. crustaceans; Group 1 in Wiggin et al., 1980). Without the ability of active dispersal, crustaceans such as large branchiopods (Crustacea, Branchiopoda: Anostraca, Notostraca, Laevicaudata, Spinicaudata) are true residents in temporary pools, relying on dormant eggs and the egg bank in the sediment to survive through frequent catastrophes (Hairston, 1996; Brendonck and De Meester, 2003). Not being stable, egg banks can be decreased in several ways including mortality, predation, and hatching.

However, hatching may not always reach its final goal to reproduce successfully, and thus can be highly risky. Because of the stochastic hydroregime, a cohort may not be able to mature in time before a hydroperiod ends, causing the waste on egg bank (Brendonck, 1996). Under the selective pressure, large branchiopods commonly adopt asynchronous hatching, wherein hatching fractions separate into several hydroperiods rather than all concentrating in one hydroperiod (Su and Mulla, 2002; Saengphan et al., 2005; Rogers, 2014; Wang and Chou, 2015). Lowering the current hatching fraction can help to decrease the depletion of abortive hatching on egg banks, but it prevents the population from maximizing the fecundity under good conditions. This strategy is called bet-hedging, which reduces long-term fitness variation at the cost of short-term fitness optimization in stochastic environments (Seger and Brockmann, 1987; Philippi and Seger, 1989).

Although bet-hedging hatching is the common emerging pattern for large branchiopods, hatching phenology of a population should be shaped by the hydroregime of native habitat. The inclination of hatching rate should fit to the successful reproduction rate. Cohen (1966; 1968) suggested that if the probability of successful reproduction is low, hatching rate should be low accordingly. Vice versa, if chance of successful reproduction is high, hatching rate should be high. Therefore, it had been predicted to have an optimal hatching rate, which should equal to the successful reproduction rate (Simovich and Hathaway, 1997). This prediction had been demonstrated by empirical studies in anostracans (Brendonck and Riddoch, 2001; Simovich et al., 2001; Maffei et al., 2005), wherein the hatching rate was close to the long-term probability of successful reproduction or predictability of good hydroperiods. However, if a habitat has several hydroperiods during a growing season, optimal hatching rate could be complicated by bet-hedging on different scales of a hydroperiod or a growing season, and this has not been explored in large branchiopods.

Siangtian Pond is a temporary pool of Taiwan, characterizing by the significant fluctuation between inundation and drought (Huang et al., 2010). Previous study on the hatching phenology of fairy shrimp *Branchinella kugenumaensis* (Ishikawa, 1895) and clam shrimp *Eulimnadia braueriana* Ishikawa, 1895 demonstrated that hatching fractions skewed towards the first two to three incubations rather than separated into more incubations for both species (Wang and Chou, 2015). Will the hatching patterns of these two species of large branchiopods fit Cohen’s model (Cohen, 1966; 1968), showing the same hatching rate as the successful reproduction rate they experiences in Siangtian Pond? Additionally, *E. braueriana* displayed a generally higher hatching fraction than *B. kugenumaensis* (Wang and Chou, 2015). Will this phenomenon be reflected in the difference of successful reproduction rate?

This study aimed for testing Cohen's model on the relationship between hatching rate and successful reproduction rate of *B. kugenumaensis* and *E. braueriana* in Siangtian Pond. Although the survivorship of dormant propagules (Cohen, 1966, 1968; Ellner, 1985) and variation of successful reproduction rate (Cohen, 1968; Ellner, 1985; Tuljapurkar and Istock, 1993; Pake and Venable, 1996; Venable, 2007) have also been showed to be correlated with hatching rate, I did not consider these two factors here because dormant egg viability has not been quantified, and the hatching phenology of two populations in a single habitat (Wang and Chou, 2015) rather than including more populations across habitats with diverse hydroregimes lets the evaluation on the effect of variability in successful recruitment to be impossible. Therefore, I first reconstructed hydroperiods and successful reproduction rates based on field surveys and long-term climate data of precipitation and temperature. Then, I compared the hatching rate and successful reproduction rate under two scenarios, wherein the eggs hatch according to a hydroperiod and a year (with several hydroperiods) respectively. These two scenarios have different ranges of fitness variation. According to Cohen's model, I predicted that for both species, hatching rate would match with successful reproduction rate in either scenario. I also predicted that *E. braueriana* would have a higher successful reproduction rate than *B. kugenumaensis*. This study explored the hatching strategies of large branchiopods and discussed potential benefits and limitations in adaptation to Siangtian Pond when adopting these hatching phenologies.

Materials and Methods

Study site

Siangtian Pond is a temporary pool in Yangmingshan National Park at northern Taiwan. The basin is usually dry. After rains, especially the extreme precipitations associating with typhoons, it may be immersed in water with several meters in depth. Although its depth is relatively deeper than most of described temporary pools wherein depth is generally no more than a meter, rapid water reduction rate of 32 cm per day results in short hydroperiods of 2-3 weeks in contrast. Occasionally, further rainfalls came in time and prolonged the hydroperiod to be longer than a month (Huang et al., 2010). Three species of large branchiopods including *B. kugenumaensis*, *E. braueriana*, and *Lynceus biformis* (Ishikawa, 1895) inhabit and co-occurred frequently (Wang et al., 2012). However, each species experienced different frequencies of abortive hatching under divergent maturation time (Wang et al., 2014).

Climatic data source

Siangtian Pond does not have local weather station. Therefore, record of the nearest weather station, Anbu Weather Station which locates about 4 km northeastwardly away from Siangtian Pond, was used. I included the precipitation (mm/day) and mean air temperature (℃/day) from 1943 through 2014 into analyses, except those from 1944 through 1948 due to incomplete record of the year (n = 67).

Hydroperiod estimation

Since hydroperiod determines whether a population can reproduce successfully, I tried to use precipitation to estimate the hydroperiod. I analyzed the hydroperiods and precipitation data of the inundation episodes (n = 25) which were surveyed in 1990 and from 2004 through 2014, except 2009. I calculated the total rains (summarizing all of the rain during a given hydroperiod; mm) and total heavy rains (summarizing all of the heavy rain, i.e. > 50 mm/day following the definition of Central Weather Bureau before 1st September 2015, during a given hydroperiod; mm) respectively, and tested their linear regression with hydroperiods after log-transformation. Pearson correlation was applied (SYSTAT 12). Better explainable parameter was chose for estimating the hydroperiods.

Successful reproduction event reconstruction

During the survey periods of 1990 and of 2004 through 2014 except 2009, large branchiopods did not always appear when Siangtian Pond was inundated. The rainfall should exceed certain amounts within a few days, so the basin would be inundated with more abundant water. Even with water, they would not hatch when the temperature was improper such as the low temperature during winter. Sometimes, large branchiopods did hatch and grow in a longer hydroperiods, but they failed to reach sexual maturity due to the low temperature such as the inundation episode 2010P1 in which all of the three species did not reproduce within 23 days (Wang et al., 2014). In addition to above mentioned environmental conditions including abundant water and suitable temperature for triggering hatching and ensuring development, the hydroperiod needs to be longer than maturation time, so a population can reproduce successfully.

Therefore, I set four to five baselines including environmental conditions and developmental schedule, for sequentially determining whether each species of large branchiopods can have successful reproduction. All of the parameters of the baselines come from daily precipitation and mean air temperature record of Anbu Weather Station and life history studies on wild populations in Siangtian Pond during surveyed periods. However, hydroperiod was estimated from the equation of linear regression with total rains or total heavy rains. Because *B. kugenumaensis* and *E. braueriana* were always been observed to occur simultaneously in a given hydroperiod but *L. biformis* only appeared when the hydroperiod was longer than a week, I separated them into two groups, with *B. kugenumaensis* and *E. braueriana* as a group following the same procedure while *L. biformis* followed a different one. I used the environmental conditions wherein the longest monitored *B. kugenumaensis* occurred as the database for determining the thresholds.

Flowchart for determining the appearance, reproduction, or abortive hatching of *B. kugenumaensis* and *E. braueriana* based on life histories, long-term climate data, and reconstructed hydroperiods is shown in Figure 1. It had four baselines as the followings:

1. The minimum total rain or total heavy rains during the beginning three days of the surveyed hydroperiods in which *B. kugenumaensis* hatched (r; mm) was used as the first threshold.

1-1: $< r$: no hatch.

1-2: $\geqq r$: proceeding to Baseline 2.

2. Hydroperiod (HP; DAII: days after initial inundation) was estimated accordingly and the mean air temperature during the hydroperiod was calculated. The minimum mean air temperature in which *B. kugenumaensis* hatched during the surveyed hydroperiods (T_1; ℃) was used as the second threshold.

2-1: $< T_1$: no hatch.

2-2: ≧ T_1: proceeding to Baseline 3.

3. The minimum mean air temperature in which *B. kugenumaensis* reproduced successfully during the surveyed hydroperiods (T_2; ℃) was used as the third threshold.

3-1: < T_2: abortive hatching.

3-2: ≧ T_2: proceeding to Baseline 4.

4. Time to first clutch (*t*; DAII) was averaged for both species and used as the forth threshold respectively ($t \geqq 10$ and 8 DAII for *B. kugenumaensis* and *E. braueriana*, respectively; reanalyzing from Wang et al. (2014) plus 2014P1 wherein *t* of *B. kugenumaensis* and *E. braueriana* was 10 and 9 DAII, respectively).

4-1: $HP < t$: abortive hatching.

4-2: $HP \geqq t$: successful reproduction.

Determining procedure for *L. biformis* (Fig. 2) was a little bit different from it of *B. kugenumaensis* and *E. braueriana* on the additional threshold of *HP*, composing of five baselines as the followings:

1. The *r* during the beginning three days of the surveyed hydroperiods in which *B. kugenumaensis* hatched was used as the first threshold.

1-1: < *r*: no hatch.

1-2: ≧ *r*: proceeding to Baseline 2.

2. The *HP* was estimated accordingly. Since *L. biformis* had never been observed to

appear when HP lasted shorter than a week, HP longer than 8 days was used as the second threshold.

2-1: $HP < 8$: no hatch.

2-2: $HP \geqq 8$: proceeding to Baseline 3.

3. Mean air temperature during the HP was calculated. The T_1 in which *B. kugenumaensis* hatched during the surveyed hydroperiods was used as the third threshold.

3-1: $< T_1$: no hatch.

3-2: $\geqq T_1$: proceeding to Baseline 4.

4. The T_2 in which *B. kugenumaensis* reproduced successfully during the surveyed hydroperiods was used as the forth threshold.

4-1: $< T_2$: abortive hatching.

4-2: $\geqq T_2$: proceeding to Baseline 5.

5. The t was averaged and used as the fifth threshold ($t \geqq 15$; reanalyzing from Wang et al. (2014) plus 2014P1 wherein t of *L. biformis* was 15 DAII).

5-1: $HP < t$: abortive hatching.

5-2: $HP \geqq t$: successful reproduction.

Successful reproduction rate evaluation

Following the above mentioned determining procedures, I evaluated the successful reproduction rate (*R*; %/year) of each species during 67 years from 1943 through 2014, except 1944-1948. The *R* contained two sources of data, one from field survey of 1990 and 2004-2014 except 2009, and the rest from reconstruction basing on long-term climatic data. Because Siangtian Pond experiences a low temperature winter from December to April of the next year, hydroperiods in each year can be seen as a growing season for large branchiopods. The *R* is fluctuating rather than a fixed value across years, and it can reflect the reproductive variation that each species faces in the habitat.

Duo to neither normal distribution nor equal variance, the *R* was compared between species by Kruskal-Wallis test after bootstrapping resampling for 500 samples in 500 iterations (SYSTAT 12). Whether the difference was significant was judged by the major results of the 500 iterations. This means that in an episode (an iteration), I randomly generated *R* of each species to compose a dataset of 500 years (samples) from the original 67 years database, and compared the *R* between species throughout 500 episodes in total. Difference towards significance, insignificance, or no tendency was made based on 500 comparisons rather than a single under random effect of resampling.

Hatching rate comparison

According to the theoretical prediction (Cohen, 1966; 1968) and empirical studies (Brendonck and Riddoch, 2001; Simovich et al., 2001; Maffei et al., 2005), a population should hatch in the same ratio as the probability of successful reproduction it experiences. I tested the differences between *R* and hatching rate of *B. kugenumaensis* and *E. braueriana*. Hatching data came from repetitive indoor experiments under standard conditions (Wang and Chou, 2015). During the hatching experiments, hatched nauplii were removed and thus prevented from reproductive replenishment.

To display adaptive hatching phenology under the selective pressure of reproductive failure, large branchiopods may bet-hedge on different scales. They could bet-hedge according to a hydroperiod, showing the similar hatching rate as *R*. Alternatively, they could also bet-hedge according to a year, showing the similar cumulative hatching rate throughout several hydroperiods of a year as *R*. Hence, I compared the hatching rate with *R* in the following two scenarios:

Scenario 1. Bet-hedging on “a hydroperiod”

Large branchiopods would show the same hatching rate as *R* throughout hydroperiods, accounting for the same proportion of unhatched eggs before the start of a given hydroperiod. Figure 3A shows the schematic diagram of the resulting egg bank size after two continuous cohorts under the assumed condition. In this scenario, a population would benefit from higher fitness if it reproduces successfully in a hydroperiod, while suffer from higher risk of wasting eggs when abortive hatching happens. Consequently, fitness would vary more significantly. This is a more rewardful but risky strategy.

During field surveys, both *B. kugenumaensis* and *E. braueriana* had experienced two continuous abortive hatchings from 2010P1 through 2012P1 (Table 2 in Wang et al., 2014). It means that they showed three continuous hatching without any replenishment during the same period. Therefore, I used the synonymous data of hatching rates ranged from the 1st through the 3rd hydroperiod in Wang and Chou (2015) for comparing with the evaluated R. Since the hatching rate here is the hatching fraction of the current egg bank rather than the definition of Wang and Chou (2015) as the hatching proportion of the original egg bank, I recalculated the hatching rate (H_i; %) from Wand and Chou (2015) by

$$H_i = \frac{n_i}{N - \sum_{j=0}^{i-1} n_j} \quad (1),$$

wherein N, n_i, and n_j represent the original egg number in a given sample, hatched nauplii number in the i^{th} hydroperiod, and hatched nauplii number in the $(i\text{-}1)^{\text{th}}$ hydroperiod, respectively. I used H_1, H_2, and H_3 to compare with R.

Differences between H_i (i = 1, 2, and 3) and R in *B. kugenumaensis* and *E.*

braueriana were explored by Kruskal-Wallis test because assumptions of normal distribution and equal variance were not met, after bootstrapping resampling for 500 samples in 500 iterations (SYSTAT 12). This method randomly simulated H_i in 500 hydroperiods, mimicking stochastic hatching over a range of fractions, and compared with R for 500 episodes. Significance or not was conclude from the majority of these 500 episodes.

Scenario 2: Bet-hedging on "a year"

In this scenario, large branchiopods would show the same hatching rate as R across years. In other words, the cumulative hatching rate throughout several hydroperiods of a year would account for the same proportion of original eggs in a given year, wherein the hatching amounts are separated evenly into these hydroperiods. The schematic diagram of the resulting egg bank size after two continuous cohorts under the assumed condition is shown in Figure 3B. Although the fitness when a population reproduces successfully is not as high as in Scenario 1, abortive hatching also causes less serious decrease on egg bank size. As a result, fitness fluctuates within a relatively smaller range, thus this strategy is more conservative.

If there averaged k^{th} hydroperiods after including both field survey and hydroperiods reconstruction from long-term climate data, I cumulated the hatching rate

(CH_i; %) until the k^{th} hydroperiod from data of Wang and Chou (2015) for comparison with *R*.

Differences between CH_i ($i = k$) and *R* in *B. kugenumaensis* and *E. braueriana* were compared by Kruskal-Wallis test because of violating basic assumptions of normal distribution and equal variance, after bootstrapping resampling for 500 samples in 500 iterations (SYSTAT 12). This treatment simulates CH_i for 500 years, providing a range of stochastic hatching fractions for comparison in 500 episodes. Deviation from *R* was made by the main results of these 500 episodes.

Results

Hydroperiod estimation

Total heavy rains showed a significant correlation with hydroperiods ($r^2 = 0.83$, $p < 0.001$; Fig. 4), and was better explainable than the correlation between total rains and hydroperiods ($r^2 = 0.82$, $p < 0.001$) during the surveyed periods. Therefore, I used total heavy rains to estimate the hydroperiod by

$$\ln^{\textit{hydroperiod}} = 0.8607 \times \ln^{\textit{total heavy rain}} - 2.7592 \text{ (2),}$$

in which the unit of hydroperiod and total heavy rain were DAII (days after initial inundation) and mm, respectively. Referring to application, it is also easier to distinguish the start of a hydroperiod by occurrence of heavy rains in short-term and to summarize them due to the generally low frequencies.

To reconstruct the successful reproduction event of large branchiopods in Siangtian Pond, the first baseline I used was the minimum total heavy rains (r; Fig. 1 and Fig. 2) during the beginning three days of the surveyed hydroperiods wherein *B. kugenumaensis* appeared. The threshold r was 127.8 (mm/the beginning three days) in 1990P3. If r was higher than this value, I applied Equation 2 to estimate the hydroperiod. If there had more heavy rains happened during the estimated hydroperiod, they were summarized into total heavy rains and re-calculated the hydroperiod until there was no more heavy rain during an estimated hydroperiod. The following thresholds of minimum mean air temperature in which *B. kugenumaensis* hatched (T_1; ℃) and reproduced successfully (T_2; ℃) during the surveyed hydroperiods were 14.7 and 15.9 in 1990P1 and 2004P4, respectively.

Successful reproduction rate evaluation

The successful reproduction rate (*R*) of *B. kugenumaensis*, *E. braueriana*, and *L. biformis* was 47.4 ± 35.2, 53.9 ± 34.4, and 44.7 ± 38.1 (%/year), respectively (Fig. 5). Difference was insignificant between *B. kugenumaensis* and *L. biformis* (Kruskal-Wallis, $p \geqq 0.05$ in 461 of the 500 iterations), while it had no tendency between *B. kugenumaensis* and *E. braueriana* (Kruskal-Wallis, $p \geqq 0.05$ in 251 of the 500 iterations) and between *E. braueriana* and *L. biformis* (Kruskal-Wallis, $p \geqq 0.05$ in 251 of the 500 iterations). Overall, *E. braueriana* had a lower dispersion of *R*, reflecting in its coefficient of variation (CV = 0.61) comparing to both *B. kugenumaensis* and *L. biformis* (CV = 0.71 and 0.78 for *B. kugenumaensis* and *L. biformis*, respectively).

Hatching rate comparison

Scenario 1. Bet-hedging on “a hydroperiod”

Hatching rate (H_i, $i = 1, 2,$ and 3) averaged 13.4 ± 13.8 % and 25.4 ± 18.7 % for *B. kugenumaensis* and *E. braueriana*, respectively. Difference between H_i and R was significant in both *B. kugenumaensis* (Kruskal-Wallis, $p < 0.001$ for all of the 500 iterations) and *E. braueriana* (Kruskal-Wallis, $p < 0.001$ for all of the 500 iterations; Fig. 6).

Scenario 2. Bet-hedging on “year”

From hydroperiod estimation, Siangtian Pond averaged 2.9 ± 1.4 hydroperiods per year, so we cumulated the hatching rate until the 3rd hydroperiod (CH_3) to compare with R in this scenario. The CH_3 was 35.1 ± 14.3 % and 58.5 ± 18.8 % for *B. kugenumaensis* and *E. braueriana*, respectively. It was significantly different with R in *B. kugenumaensis* (Kruskal-Wallis, $p < 0.05$ in 498 of 500 iterations), while the difference was insignificant in *E. braueriana* (Kruskal-Wallis, $p \geqq 0.05$ in 458 of 500 iterations; Fig. 7).

To summarize, differences of R were not significant between species, but *E. braueriana* showed a lower CV. Although hatching rate of *B. kugenumaensis* was significantly different with R in both scenarios, it of *E. braueriana* was insignificantly different with *R* in Scenario 2, showing similar hatching fractions of the original egg bank in a year as the long-term experienced *R* under Siangtian Pond hydroregime.

Discussion

Facing the frequent unfavorable periods in temporary pools, maintaining the resistant dormant eggs is key to the persistent of large branchiopods (Brendonck and De Meester, 2003). Hatching strategies are important for not only recruiting eggs but also reducing the depletion from abortive hatching. To clarify the bet-hedging hatching can help to explore the adaptive hatching strategy of populations to local habitats.

Combining field survey on the life history and climate data including precipitation and temperature, I estimated the hydroperiods and successful reproduction rates which large branchiopods experienced in Siangtian Pond. This method provides a way to test Cohen's model (Cohen, 1966; 1968) on the consistency between hatching rate and successful reproduction rate. Because total heavy rains explained only 82 % of variation in hydroperiods and maturation time of each species varied within a range, underestimation and overestimation on successful reproduction rate would happen. Nevertheless, variation of successful reproduction rate can be revealed and provided the information about reproductive uncertainty. The highly varied probability of successful reproduction ranged from 0 through 100 per year for all of the three species. It may mask the differences between species which have separated maturation schedules, rejecting my prediction that *E. braueriana* would have a higher successful reproduction rate than *B. kugenumaensis*.

To estimate hydroperiods of Siangtian Pond, I found that amount of heavy rains, i.e. more than 50 mm per day, was a good predictor. Furthermore, the occurrence of heavy rains within a short period of three days over a certain level could indicate the start of a hydroperiod. These may cause by Siangtian Pond characteristic hydroregime wherein the water comes from precipitation only, especially accompanying with the torrential rains bringing by typhoons (Wang and Chou, 2015). If large branchiopods can use the environmental factors relating with abundant water such as the reduction of conductivity (Brendonck et al., 1998; Vanschoenwinkel et al., 2010) as reliable signals, the stochasticity of Siangtian Pond hydroregime lies more on hydroperiods than inundations, in which the former are unstable but the latter are expected after heavy rains.

Referring to hatching phenology, my results showed that in Scenario 2, the cumulative hatching rate throughout the first three incubations was not significantly different from successful reproduction rate in *E. braueriana*, while the difference was significant in *B. kugenumaensis*. In Scenario 1, hatching rates of the same incubating periods were significantly different from successful reproduction rates in both species. My prediction that hatching rate would fit to successful reproduction rate only worked in *E. braueriana* under Scenario 2, but not in *B. kugenumaensis*. It means that *E.*

braueriana bet-hedged the eggs according to a year, but not to a hydroperiod. Although both scenarios act as bet-hedging strategy and have the same fitness in average, the resulting variation of fitness are different. Following Scenario 1 and bet-hedging on each hydroperiod, large branchiopods may achieve higher fitness in a current cohort. Nevertheless, they also suffer from more loss when abortive hatching happens. On the other hand, adopting Scenario 2 and bet-hedging on each year, allows lesser fitness cost in abortive hatchings, even though the fitness is lower when they meet favorable periods. As what bet-hedging proposes, under the tradeoff between maximizing mean fitness and minimizing fitness variation, selection would favor the strategy which reduces the variation of fitness even at the cost of mean fitness optimization in stochastic environments (Seger and Brockmann, 1987; Philippi and Seger, 1989).

Hatching strategy of *E. braueriana* seems to be satisfied with the conservative bet-hedging, which avoids the extreme in fitness (Philippi and Seger, 1989) by lowering current hatching and offspring number (Maffei et al., 2005). Additionally, it should be mentioned that hatching fractions of *E. braueriana* were not evenly distributed across these three incubations, with the higher hatching rate in the second incubation (Wang and Chou, 2015). This kind of unfixed germinating rate has also been demonstrated in plants (Philippi, 1993; Clauss and Venable, 2000; Evans et al., 2007). Since the large-scale rains mostly happened from August through October and created a second peak of inundations, the uneven hatching fractions distribution could synchronize *E. braueriana* with Siangtian Pond hydroregime and recruit more eggs (Wang and Chou, 2015). In other words, the distribution of hatchings is “predictive” of hydroregime, correlating with future reproductive success (predictive/adaptive phenotypic plasticity, Evans et al., 2007; Simons, 2014). Although hatching plasticity does not belong to bet-hedging strategy (Evans et al., 2007), the coexistence of both adaptive strategies is not in conflict (Simons, 2014).

Hatching rates of *B. kugenumaensis* were not fitted with successful reproduction rate in both scenarios. In general, its hatching rate was lower than expected, optimal hatching fraction. The dissimilarity to what bet-hedging predicts may be that Siangtian Pond hydroregime does not select for an optimal hatching strategy in *B. kugenumaensis*, as what Philippi (1993) suggested to the variations of germinating behaviors of winter annuals. *Branchinella kugenumaensis* may not totally rely on hatching to survive in Siangtian Pond, lowering the selective pressure on an optimal hatching strategy. But I can not exclude the possibility of physiological restriction on adjusting hatching responses (Clauss and Venable, 2000). If I assume that all of the viable eggs hatched after the repetitive hatching experiments so that the remaining eggs were not in prolonged dormancy, the low hatching rate of *B. kugenumaensis* may result from egg mortality. Maybe *B. kugenumaensis* eggs are less resistant to desiccation or the preservation conditions, and this physiology of eggs limits the optimal hatching response.

However, the egg bank size of *B. kugenumaensis* had been demonstrated to be close to *E. braueriana* at the similar numerical magnitude (Wang et al., 2014). Although Wang et al. (2014) did not evaluate the viability of eggs and thus the egg bank size may contain the dead eggs, it still indicate that *B. kugenumaensis* has other ways to compensate the inferiority in hatching strategy and resulted in the increase of egg bank based on the fitness evaluation through the four cohorts of 2005 (Huang et al., 2011). Previous study found that the mean daily density of adult *B. kugenumaensis* was higher than *E. braueriana*, even up to tenfold (Chapter 2), so it is reasonable to infer that *B. kugenumaensis* generally had more abundant population size than *E. braueriana*. Accompanying with lower hatching rate, dominance in free-living population of *B. kugenumaensis* implies higher survivorship before developing into adults. It is also possible that the clutch size of *B. kugenumaensis* and the resulting fecundity per cohort are higher than *E. braueriana*. Moreover, *B. kugenumaensis* displayed alternative growth patterns between determinate and indeterminate growth according to hydroperiods (Huang et al., 2010). Body length differed both among cohorts and within cohorts. Because clutch size was highly correlated with body length, fitness may also vary significantly among individuals of the same cohort. It is likely that *B.*

kugenumaensis adopts diversified bet-hedging, increasing fitness variation among individuals within generations (Philippi and Seger, 1989).

Combining the life histories and hatching phenologies of *B. kugenumaensis* and *E. braueriana*, each species may take different strategies to live in Siangtian Pond. *Eulimnadia braueriana* has low density and size in active population (Chapter 2), and shorter life span, almost disappearing after the third weeks of a hydroperiod (Wang et al., 2012). Facing several limitations on free-living stage, more conservative hatching helps *E. braueriana* to decrease the cost from abortive hatching. Differently, hatching fraction of *B. kugenumaensis* did not optimize, reaching the ratio as its successful reproductive probability. Nevertheless, generally more abundant active population sizes (Chapter 2), flexible growth patterns (Huang et al., 2010), and longer longevity (Wang et al., 2012), provide the free-living stage of *B. kugenumaensis* with several ways to take advantage of Siangtian Pond hydroregime opportunistically. That is to say, *E. braueriana* bet-hedges on the hatching of dormant eggs, while *B. kugenumaensis* bet-hedges on the life history of free-living individuals.

Lynceus biformis never hatches during the standard culture conditions in which I rear *B. kugenumaensis* and *E. braueriana* successfully, so my understanding on its life history and hatching phenology is quite limited. No appearance when the hydroperiod was shorter than a week (Wang et al., 2014) means that *L. biformis* could either need longer time to initialize hatching, or its eggs are more sensitive to the environmental cues and only hatch when Siangtian Pond has abundant water. Although delayed occurrence during a hydroperiod prevents *L. biformis* from abortive hatching in the short hydroperiods, it still has the risk of abortion if the hydroperiod ends before *L. biformis* matures. Thus, successful reproduction rate of *L. biformis* also fluctuated significantly. Variation in successful reproduction rate of *B. kugenumaensis* and *E. braueriana* mainly comes from less selective hatching to the hydroperiod, while it of *L. biformis* may relate to late occurrence and maturation. The especially abundant egg bank size of *L. biformis* (Wang et al., 2014) may indicate the other adaptive strategies of active population such as fecundity, which needs more field surveys and the breakthrough in its indoor culturing to clarify.

In conclusion, successful reproduction rate, which was reconstructed from field surveys and long-term climate data, fluctuated significantly across the years for all of the three species of large branchiopods in Siangtian Pond. To test Cohen’s model, I found that only *E. braueriana* showed the similar cumulative hatching rate throughout the first three hydroperiods to its successful reproduction rate, while the hatching rate of *E. braueriana*, and both the hatching rate and cumulative hatching rate of *B. kugenumaensis* were inconsistent with successful reproduction rate. *Eulimnadia braueriana* displayed a more conservative hatching, bet-hedging the eggs on a year rather than a hydroperiod. This further enhances the reduction in fitness variation at the cost of optimizing the fitness in current cohort. Although *B. kugenumaensis* had a low hatching fraction than expected, life histories such as flexible growth patterns may help it to take advantage of unstable hydroperiods opportunistically. Overall, *E. braueriana* may rely more on hatching strategies to decrease the depletion from abortive hatchings, but *B. kugenumaensis* on life history strategies of free-living individuals to balance the weakness of hatching performance.

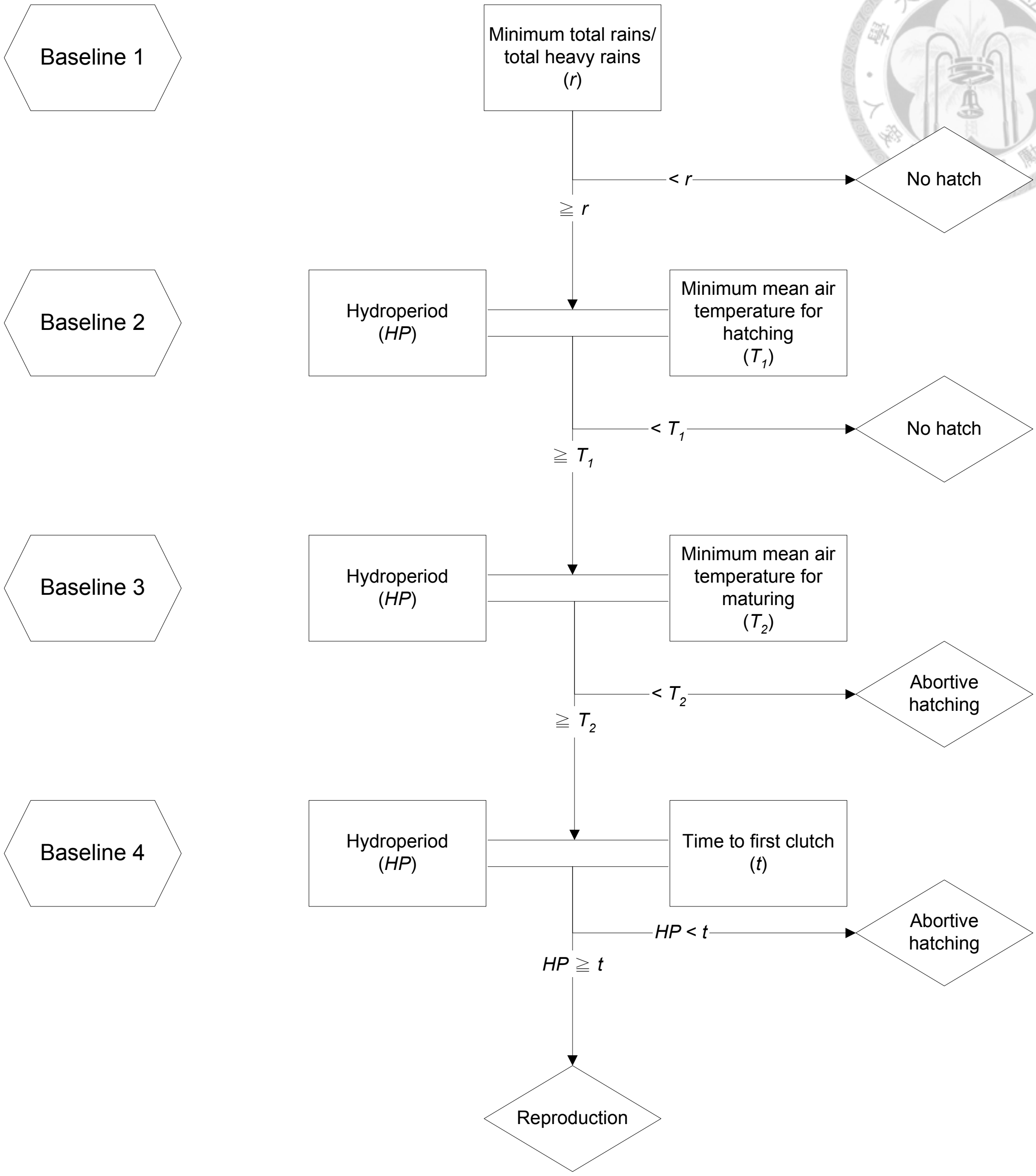

Figure 1. Flowchart for determining the appearance, reproduction, and abortive hatching of *B. kugenumaensis* and *E. braueriana*.

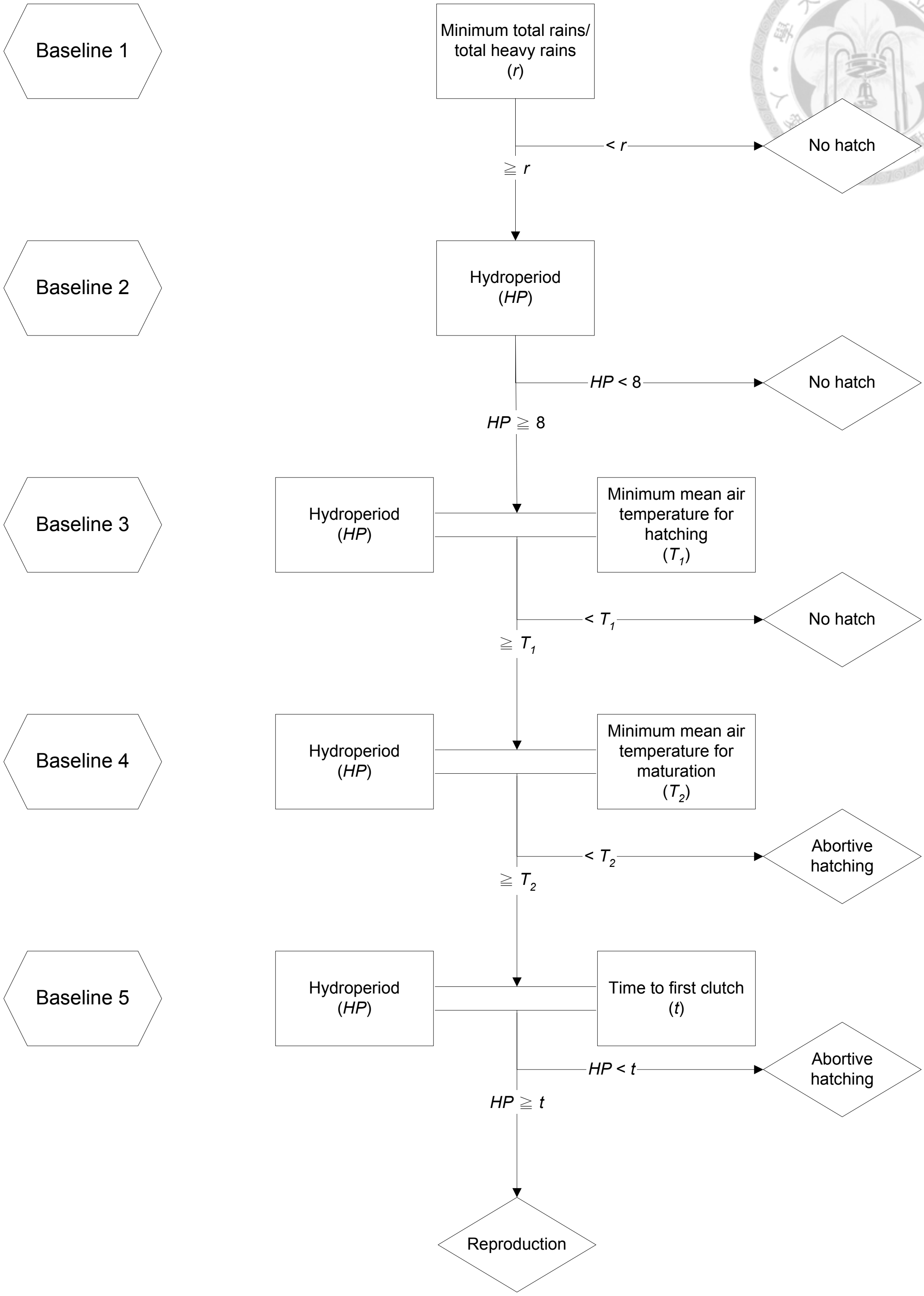

Figure 2. Flowchart for determining the appearance, reproduction, and abortive hatching of *L. biformis*.

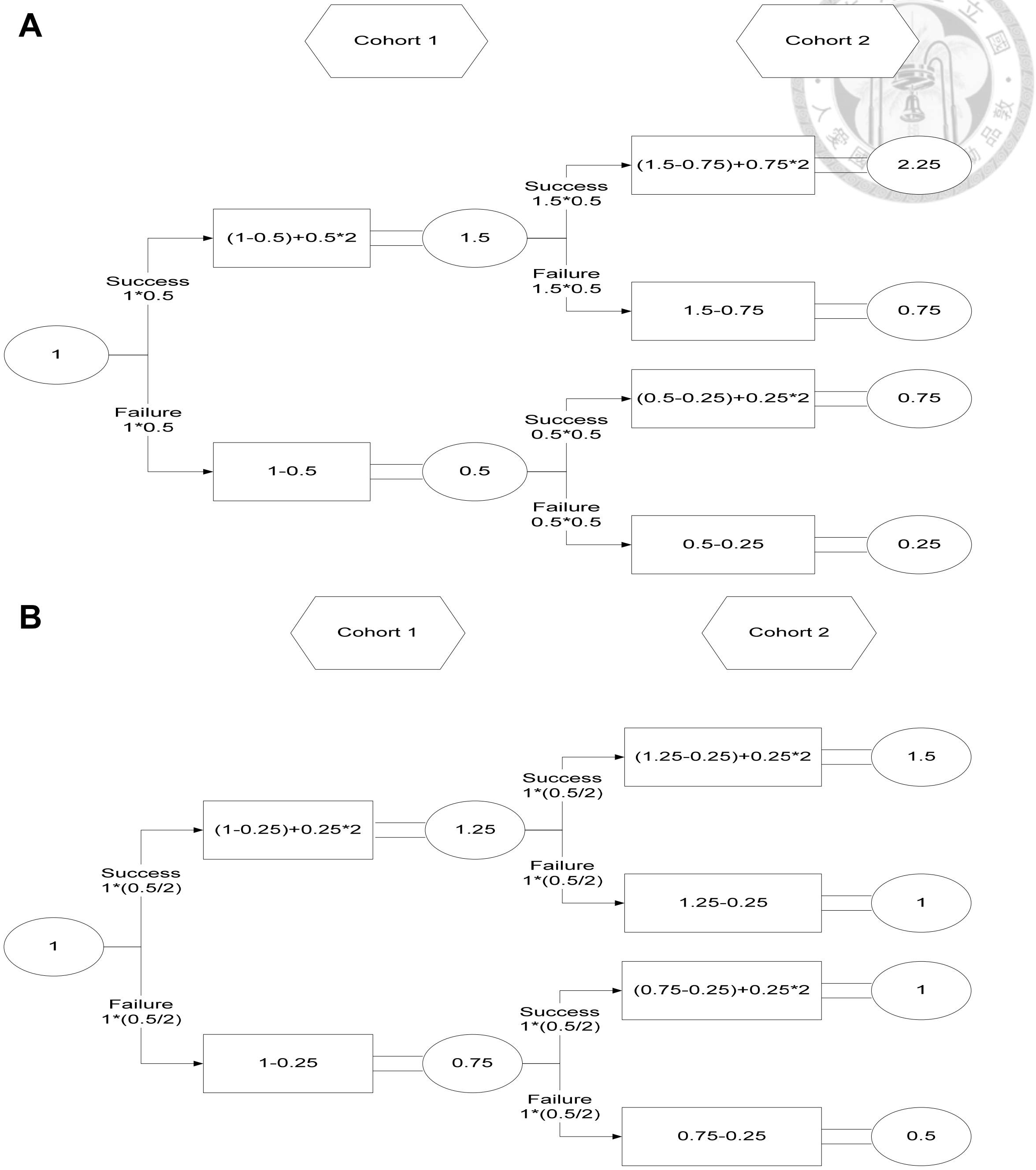

Figure 3. Diagram of the egg bank size, comparing with the origin, after two continuous cohorts under (A) Scenario 1 and (B) Scenario 2. General assumptions: original egg bank size is one; each hatched egg will replenish two eggs back to the egg bank if the cohort can reproduce successfully (the main result of Huang et al., 2011); no egg mortality exists. Scenario 1 assumption: a population will hatch 50 % of its current

eggs in each hydroperiod. Scenario 2 assumption: a population will hatch 50 % of its original eggs in a year with two hydroperiods, and 25 % of its original eggs in each hydroperiod.

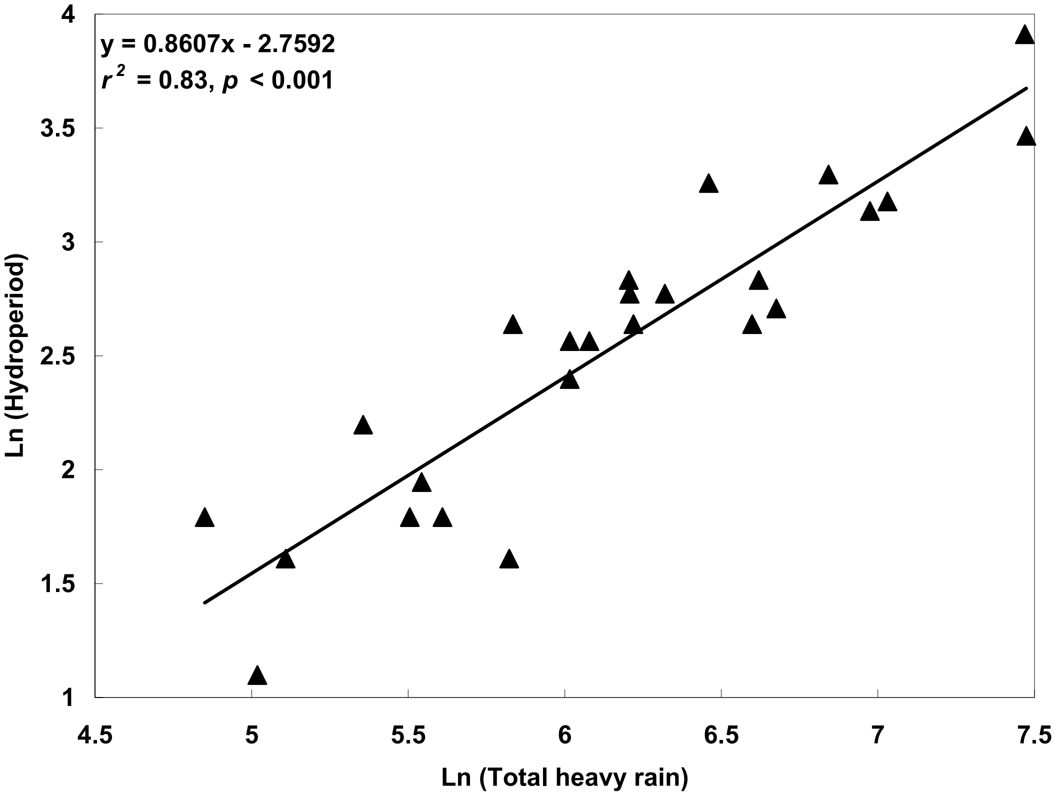

Figure 4. Linear regression between total heavy rains and hydroperiods after log-transformation.

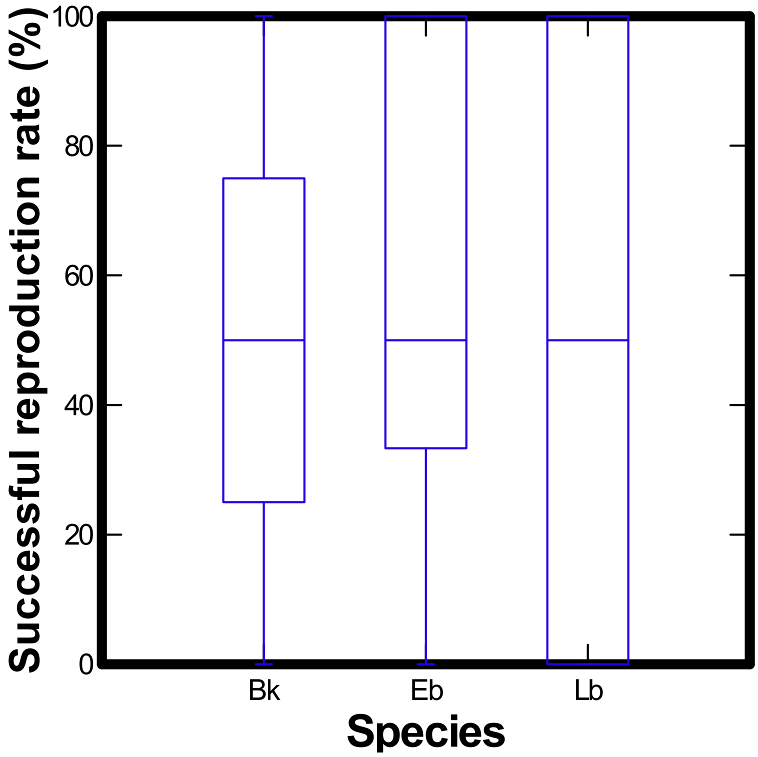

Figure 5. Successful reproduction rate of *B. kugenumaensis* (Bk), *E. braueriana* (Eb), and *L. biformis* (Lb). Box plot shows the range, first IQR (interquartile range), median, and third IQR.

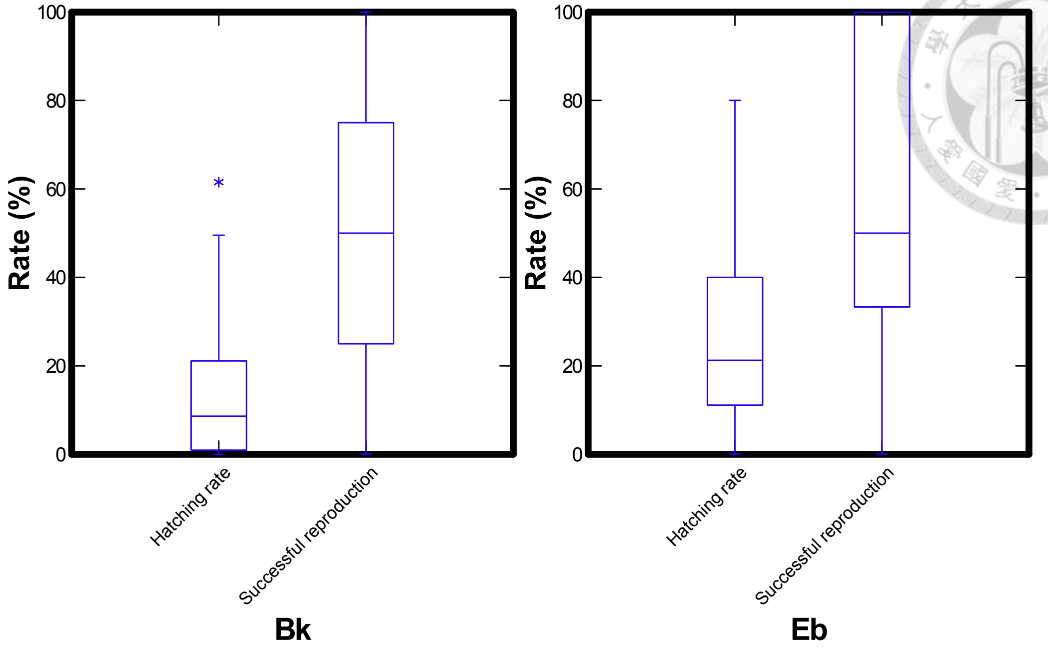

Figure 6. Box plots show the range, first IQR (interquartile range), median, third IQR, and extreme value of the hatching rate and successful reproduction rate of *B. kugenumaensis* (Bk) and *E. braueriana* (Eb). The difference was significant in both species under Scenario 1 in all of the 500 iterations.

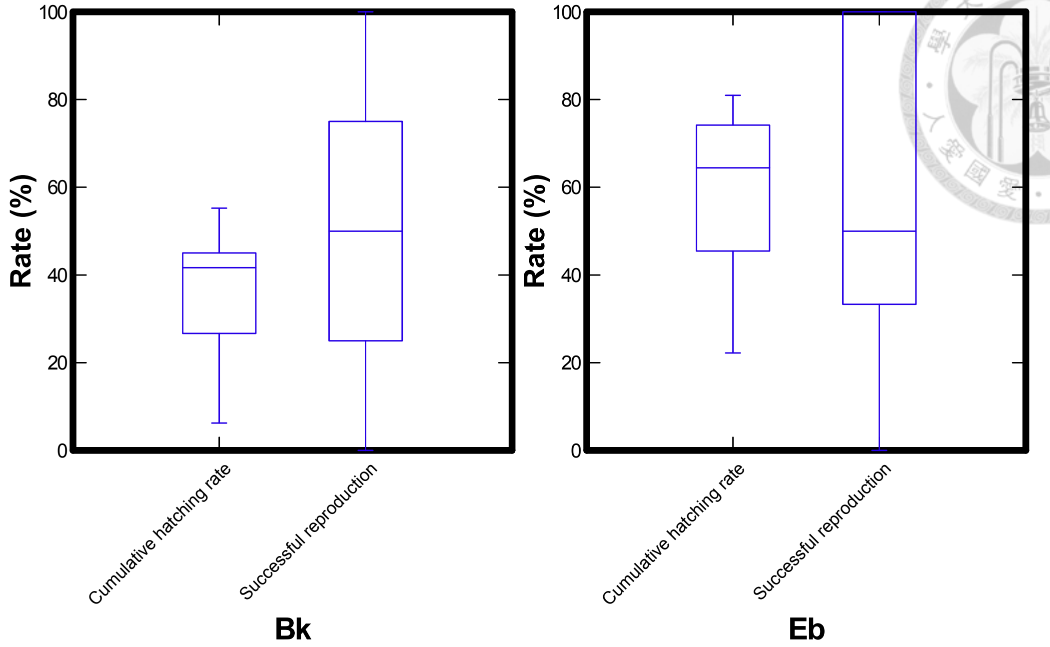

Figure 7. Box plots show the range, first IQR (interquartile range), median, and third IQR of the cumulative hatching rate (*CH_3*) and successful reproduction rate of *B. kugenumaensis* (Bk) and *E. braueriana* (Eb). The difference was significant in *B. kugenumaensis* in 498 of the 500 iterations, but was insignificant in *E. braueriana* in 458 of the 500 iterations under Scenario 2.

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Chapter 6: General conclusion

Referring to the spatial utilization of large branchiopods *Branchinella kugenumaensis*, *Eulimnadia braueriana*, and *Lynceus biformis* in Siangtian Pond at northern Taiwan, significant spatial differentiation had been demonstrated both in horizon and vertical aspects. This dissertation further focused on horizontal patterns of free swimming adults and eggs. I found hotspots of *B. kugenumaensis* and *L. biformis* located centrally near the three deepest places of Siangtian Pond, while *E. braueriana* hotspots dispersed widely across the basin, with inclination towards the eastern longitudinal path. As for the egg bank, *B. kugenumaensis* eggs were mainly distributed within the three deepest places. Eggs of *E. braueriana* also clumped around the artificial path, but the inclining tendency was towards the northwest. *Lynceus biformis* eggs were widespread in the southern part of Siangtian Pond, even reaching the most peripheral regions.

Some consistency and inconsistency appear between the distribution of adults and eggs. For example, the pattern was consistent in *B. kugenumaensis*, with the hotspots locating in the three deepest places of Siangtian Pond, which means that spatial structure of *B. kugenumaensis* egg bank was mainly determined by long term habitat utilization of adults. However, a contradiction happens in *E. braueriana* and *L. biformis*.

For *E. braueriana*, the hotspots inclined towards the east and west in adults and eggs respectively, and for *L. biformis*, while adults was distributed centrally in the three deepest places, its eggs were widely scattered across the southern basin and reached the edge of Siangtian Pond. This kind of inconsistency implies that redispersal may work after the eggs are laid. Potential mechanisms such as wind blowing and egg flotation could operate independently or interactively and further disperse the eggs away from original regions. As a whole, each species faces different opportunities and limitations from edge aggregation, development, available inundation area, and egg redispersal for achieving optimal edge distribution of the egg, complicating the spatial interactions between free swimming individuals and dormant eggs.

On the other hand, hatching phenology plays a key role in the linkage between free swimming adults and dormant eggs, and should be shaped by the long term hydroregime of habitat. From laboratory hatching experiments, I found that both *B. kugenumaensis* and *E. braueriana* hatched in the early days of a given hydroperiod, and the majority of hatching fractions occurred in the early two to three hydroperiods. Further exploration of whether the hatching rate is as optimal as the prediction by bet hedging strategy, results showed that only the cumulative hatching rate of *E. braueriana* fitted for its successful reproduction rate, while no match was found for *B. kugenumaensis*. Accordingly, I suggest that *E. braueriana* adopts a more conservative hatching strategy, but *B. kugenumaensis* does not hatch optimally.

Looking into the life cycle of *B. kugenumaensis* and *E. braueriana*, both species may survive by different ways. With a smaller population size and a shorter longevity, *E. braueriana* may suffer from a lower fecundity and rely on hatching strategy to reduce potential loss of abortion. Differently, *B. kugenumaensis* displays a deficient hatching, which may offset by a more abundant population size, a longer life span, and flexible patterns between determinate and indeterminate growth, to opportunistically increase the recruitment of each cohort. Overall, it seems like that *E. braueriana* acts as a conservative bet hedger in hatching of dormant eggs, but *B. kugenumaensis* behaves as a diversified bet hedger in reproducing of free swimming adults to live in the temporary Siangtian Pond.