國立台灣大學生命科學院生態學與演化生物學研究所

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真湖蚌蟲趨光性及攝食形態的發育變異

Ontogenic changes in phototaxis and feeding morphology of the clam shrimp *Eulimnadia braueriana* Ishikawa, 1895

劉哲聿

Jer-Yuh Liu

指導教授：周蓮香 博士

Advisor: Lien-Siang Chou, Ph.D.

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摘要

趨光性會影響生物的分布而型態能顯示其功能，本研究以不同照度的人造光源測試真湖蚌蟲 (*Eulimnadia braueriana* Ishikawa, 1895) 無節幼體、幼體及成體階段的趨光性，並以光學及掃描式電子顯微鏡觀察其攝食構造的形態發育，以探索此物種對於分布和取食的偏好。雖然蚌蟲在發育過程中外觀會有明顯變化，但過去少有探討其發育過程至成熟期的研究。實驗結果顯示，真湖蚌蟲於無節幼體階段會表現出正趨光性，但進入幼體期之後會轉為負趨光性。形態的轉變也大約在同樣時期，主要攝食構造由第二對觸角和大顎轉為較尾端的胸足、小顎、成熟大顎的組合。其攝食構造大多符合濾食性物種的特徵，但成體在前幾對胸足末端具有較堅固的梳狀構造，推測可能也具有刮食能力。從趨光性的結果可以推斷真湖蚌蟲於無節幼體期白天會游向水面，在有攝食能力後以浮游藻類及懸浮顆粒為食。進入幼體期後，真湖蚌蟲將因負趨光性聚集於水底，攝食方式可能濾食由底層懸浮或刮起的物質。在發育過程中的趨光性變化會造成生物的遷徙，可能可以幫助此物種更均勻分布於棲地中。此外，比較向天池三種共域大型鰓足類成體的形態發現，真湖蚌蟲分別與鵠沼枝額蟲 (*Branchinella kugenumaensis* Ishikawa, 1895) 及貓眼蚌蟲 (*Lynceus biformis* Ishikawa, 1895) 有相似形態，可能在食性上有競爭壓力；貓眼蚌蟲和鵠沼枝額蟲間則在形態上有明確差異，推測應有棲位分化現象。

關鍵字：照度、功能形態學、陽明山國家公園、大型鰓足類、無甲目、平尾目。

Abstract

Phototaxis influences distribution and morphology is representative of function. While clam shrimps undergo an apparent change in appearance during development, ontogenic studies until maturation were seldom the center of attention. In this study, phototaxis through the nauplius, juvenile and adult stages with artificial lighting of different illuminances were tested and developmental morphology of feeding structures were observed with both light microscopy and SEM to explore their preference for general distribution and food. Results revealed that they first show positive phototaxis at the nauplius stage while changing into negative phototaxis from the juvenile stage on. Morphological change and a posterior shift in the main feeding structures also happen at the juvenile stage. The main feeding structures change from the second antenna and mandibles to the more posterior combination of sophisticated mandibles, maxillae and thoracopods. While the feeding structures mostly fit the description for filter feeding animals, scraping structures have also been found on the first several thoracopod pairs in the adult stage. The results imply that newly hatched *E. braueriana* nauplii will swim to the water surface during day time and filter feed on planktonic particles once having the ability to feed. Reaching the photo-negative juvenile stage, they may gather at the bottom, likely filtering suspended or scraped up particles as food source from this stage on. Ontogenic change in phototaxis leads to migration which could help this species distribute more even through the habitat, avoiding intraspecific competition. In addition, comparing adult feeding structures with the sympatric large branchiopods Siangtian Pond revealed potential competition for *E. braueriana* with the other two species, *Branchinella kugenumaensis* Ishikawa, 1895 and *Lynceus biformis* Ishikawa, 1895, and suggested resource partitioning between *B. kugenumaensis* and *L. biformis*.

Key words: illuminance, functional morphology, Yangmingshan National Park, large branchiopod, Anostraca, Laevicaudata.

Index

摘要.. I

Abstract II

Chapter 1: Grand Introduction 1

References 4

Chapter 2: Ontogenic change in phototaxis of the clam shrimp *Eulimnadia braueriana* Ishikawa, 1895 (Branchiopoda: Spinicaudata)

Abstract 7

Introduction 8

Material and Methods 12

Results 15

Discussion 16

References 23

Table and Figures 29

Chapter 3: Developmental and functional morphology of *Eulimnadia braueriana* Ishikawa, 1895 feeding structures with comparison of adult sympatric large Branchiopoda (Crustacea)

Abstract 33

Introduction 35

Material and Methods 38

Results 40

Discussion 49

References 56

Figures 61

Chapter 4: Grand Conclusion 79

Appendix 1 81

Appendix 2 83

Tables

Table 1: 29

Figures

Figure 2-1 30

Figure 2-2 31

Figure 2-3 32

Figure 3-1 61

Figure 3-2 62

Figure 3-3 63

Figure 3-4 64

Figure 3-5 66

Figure 3-6 68

Figure 3-7 69

Figure 3-8 70

Figure 3-9 71

Figure 3-10 72

Figure 3-11 73

Figure 3-12 74

Figure 3-13 76

Figure 3-14 77

Chapter 1: Grand introduction

Branchiopoda is a taxon within the class Crustacea, unified by the feature that gills are present on the base of the appendages (Cuvier, 1851). The non-scientific term "large branchiopods" is used for convenience to refer to the orders Anostraca, Notostraca, Laevicaudata, Spinicaudata and Cyclestherida (Rogers, 2009). They can be found around the globe except for the Arctic and show wide variety of adaptations (Brendonck et al., 2008). Large branchiopods mostly inhabit freshwater temporary wetlands, with exception of a few genera that need saline environments (Brendonck et al., 2008). Temporary wetlands encompass any environment that dries out or freezes solid and refills with liquid water over a course of time. Inundation sources range from precipitation, flooding, snow-melt, even to artificial irrigation (Brooks, 2000; Grygier et al., 2002; Eder and Hödl, 2002). Some of these habitats are highly stochastic (Brooks, 2000; Bauder, 2005). In order to persist in such unstable environment, large branchiopods have a quick life cycle (Hildrew, 1985; Brendonck *et al.*, 2000; Huang et al., 2010), produce resting eggs to endure the dry periods (Brendonck and De Meester, 2003) and employ a bet hedging strategy at hatching to minimize the damage of abortive hatching (Simovich and Hathaway, 1997; Ripley et al., 2004).

Siangtian pond, located in Yangmingshan National Park, Taipei, Taiwan, is an ephemeral pond at 818 m above sea level (Huang et al., 2010). Combining the water loss from evaporation and leaking due to porous substrate, water depth reduces at a rate of approximately 32 cm per day (Huang et al., 2010). Intense rainfalls within a short time period are needed to create an inundation period long enough for the large branchiopods to reproduce. This mostly occurs from monsoon rain around May and typhoons in summer and early autumn (Huang et al., 2011). There are three large branchiopod species inhabiting Siangtian pond: *Branchinella kugenumaensis* (Ishikawa, 1895), *Lynceus biformis* (Ishikawa, 1895) and our species of interest, *Eulimnadia braueriana* Ishikawa, 1895 (Wang et al., 2012). Previous research on these three species in Siangtian pond have revealed their community structure (Huang et al., 2008), mitochondrial DNA identification (Huang and Chou, 2011), spatial niche differentiation (Wang et al., 2012), egg bank dynamics (Wang et al, 2014) and hatching phenology (Wang and Chou, 2015). Studies on individual species include the general description (Lin and Chou, 1991), population dynamics (Huang and Chou, 2005), life history (Huang et al., 2010) and reproduction potential (Huang et al., 2011) of *B. kugenumaensis*, and the life history under different temperatures in *E. braueriana* (Huang and Chou, 2015). But among these studies, behavior and morphological studies were few and their nauplius and juvenile stage needed more attention.

Eulimnadia braueriana is a clam shrimp of the order Spinicaudata. In a temporary pond, it will experience drastic environmental change and also undergoes significant alteration in appearance through its development as a clam shrimp. Different developmental stages might have different behavior and preferences. Being sympatric with two other large branchiopods further raises the question of how they coexist in this environment. In this study, information on their development and life strategies is gathered. Phototaxis is an innate trait that tells us about their general swimming pattern and preference for distribution. So the first aim is to know the phototaxis of *E. braueriana* at different illuminance and developmental stages.

There is a variety of factors that are directly or indirectly related to the causes or consequences of phototaxis. Among them, feeding is a trait that is not only indispensable through its whole life, unlike reproduction or dormancy happening only at certain stages, it is also not restricted to population or environment, unlike predator avoidance. How to handle and ingest food can be inferred from the morphology of the responsible structures (Watling and Thiel, 2013). So the second aim is to follow the developmental morphology of their feeding structures. Combining phototaxis and feeding ability is able to give an overview on the life of *Eulimnadia braueriana*. Further comparing feeding structure morphology with the traits of the other two sympatric large branchiopods, we get a broader view on the large branchiopod ecology in Siangtian pond.

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Chapter 2: Ontogenic change in phototaxis of the clam shrimp *Eulimnadia braueriana* Ishikawa, 1895 (Branchiopoda: Spinicaudata)

Jer-Yuh Liu, Chun-Chieh Wang, Lien-Siang Chou

Abstract

Phototaxis is an important behavior during the life cycle of branchiopods, affecting survivorship and reproduction. But few studies have traced it through development and it has not been recorded in Spinicaudatans. In this study, photoresponse across nauplii, juveniles and adult hermaphrodites of the clam shrimp *Eulimnadia braueriana* was measured under four different artificial illuminances ranging from 19,688 to 48,286 lx. Our results demonstrate that the nauplii displayed consistent positive phototaxis through all illuminance ranges. The juveniles and adults, however, showed no response to the dimmest light and reacted negatively at all the other treatments. The change in phototaxis occurs when nauplii develop into juveniles and may lead to a different vertical distribution. It signifies an adaptive strategy in dispersal, foraging, predator avoidance and minimizing intraspecific competition.

Key Words: illuminance, large branchiopod, distribution.

Introduction

Phototaxis plays an important role in survival and life history in a wide range of species, asserting both direct and indirect influences on organisms by affecting distribution, behavior and physiology (Thorson, 1964; Clyudsley-Thompson, 1966; Sorgeloos, 1972; Jekely, 2009). Organisms that have consistent negative phototaxis are usually subterranean (Schlegel *et al*., 2009; Borowsky, 2011). In such cases, eyes are mostly retrograded or substituted by other photoreceptors that are only able to perceive light but cannot create images (Schlegel *et al*., 2009; Tarttelin *et al.*, 2012). In areas with strong sunlight, negative phototaxis serves as a protection against UV radiance by letting organisms avoid areas with high radiation (Storz and Paul, 1998; Leech and Johnson, 2003). Avoidance of visual predators is also often associated with some degree of negative phototaxis (Forward and Hettler, 1992; De Robertis *et al*., 2000). Positive phototaxis, however, is far more common (Forward, 1976). In aquatic habitats, it is a guiding force towards the water surface and is found in photosynthetic organisms (Bendix, 1960; Jekely, 2009), those that feed on them, organisms that rely on vision for foraging (Spooner, 1933; Jekely, 2009), mating (Bendix, 1960), and dispersal (Thorson, 1964; Crisp, 1976).

Some species could possess both positive and negative phototaxis but exhibit only one at a time according to environmental conditions, interspecific interaction, or different stages in their life history or their physical condition (Ewald, 1912; Cushing, 1951; Hunte and Myers, 1984; Walker *et al*., 1987). Light induced migration is critical for the dispersal and settlement of many marine invertebrates with both free swimming and sessile stages (Thorson, 1964). Change of phototaxis also plays an important role in seasonal hibernation where organisms occupy different depths in the water during dormancy, as has been found in marine copepods (Martynova and Gordeeva, 2010). The above mentioned possibilities in phototaxis shift are both related to organism development and the surrounding environmental conditions. Phototaxis may also be influenced by light illuminance or wavelength (Ewald, 1912; Bendix, 1960; Menzel, 1979), such as in diel vertical migration, which is found in many crustaceans (Forward, 1988). It was the observation of similar vertical migration that started the research on phototaxis in crustaceans in the nineteenth century (Cushing, 1951).

In branchiopods, ontogenic phototaxis has been recorded in *Triops* (Davis and Madison, 2000) and revealed a phototaxis shift which was associated with selective pressures. Most research on branchiopod phototaxis was, however, done on Cladocera and Anostraca, including the effects of gender (Brendonck *et al.*, 1995), predator (Forward and Hettler, 1992; Forward and Rittschof, 1999), wavelength and intensity (Schneider and Stearns, 1998; Storz and Paul, 1998), satiation status (De Meester and

Dumont, 1989; Forward and Hettler, 1992) and genotypes (De Meester and Dumont, 1989). Phototaxis has also been found to influence swarming behavior (Clyudsley-Thompson, 1966; Villamizar *et al*., 2011) and has impacts on naupliar growth (Sorgeloos, 1972; Wei *et al*., 2014) and hatching rate (Van der Linden *et al*., 1985; Kashiyama *et al*., 2010; Villamizar *et al*., 2011). Until now, ontogenic studies on phototaxis are scarce and there were no records on Spinicaudata.

Eulimnadia braueriana Ishikawa, 1895 has been reported from the seasonally astatic wetland Siangtian pond in Yangmingshan National Park, Taiwan (N: 25˚10′26″, E: 121˚ 29′56″; Huang *et al*., 2010), where it co-occurs with *Branchinella kugenumaensis* (Ishikawa, 1895) (Anostraca) and *Lynceus biformis* (Ishikawa, 1895) (Laevicaudata) (Huang *et al*., 2010). Previous studies have shown that *E. braueriana* matured most rapidly among the three species (Wang *et al*., 2014). Their adult distribution in the wild showed fluctuating horizontal hotspots (Wang *et al*., 2012). Both vertical and horizontal distribution preferences are not clear, but we observed that *E. braueriana* nauplii congregate at the water surface while juveniles and adults are more sedentary. We hypothesized that an ontogenic shift in phototaxis occurs as food and mating requirements change with development, thus we tested *E. braueriana* phototaxis across different developmental stages. Furthermore, Siangtian Pond has highly variable depth between inundations and can reach up to 6.4 meters (Huang *et al*., 2010). Water depth creates diverse gradients of illuminations. The basin is also covered by a variety of dense vegetation and occasional trees except on two human-walked paths. Vegetation and water depth thus create an environment which complicates natural light intensity. Our second hypothesis is that illuminance is influential on *E. braueriana* distribution. Phototaxis under different artificial illuminance is therefore tested in this study.

Material and Methods

Laboratory cultivation

Eulimnadia braueriana were cultured in the laboratory using egg-laden soil collected from Siangtian pond. 30 g or 100 g such substrate was incubated in 2 L or 9.6 L water tanks respectively with aged tap water and illuminated 12 hours per day at 22-24°C. Hatched animals fed on naturally occurring algae or detritus.

Developmental stage

Individuals were categorized into nauplius, juvenile and adult stage based on their morphology (Fig. 2-1). Nauplii have neither movable thoracopods nor a bivalve carapace that covers the body laterally. The most prominent appendage in this stage is the second antennae (Fig. 2-1a). Juveniles possess movable thoracopods and a full carapace that covers the entire body when at rest (Fig. 2-1b). Adults have a similar appearance to juveniles but are larger in size and possess reproductive structures: the first two pairs of thoracopods in male are specialized as claspers, used for amplexus during mating (Fig. 2-1d); hermaphrodites have obvious, whitish eggs carried either in the ovaries (Fig. 2-1c) or in the brood pouch. Since males are relatively rare (Huang and Chou, 2015), adults used in this study were all hermaphrodites.

Phototactic experiment

Experiments were conducted in a plastic testing tank (22 × 12.5 × 5cm). The testing tank was filled to two centimeters in depth in order to limit vertical movement, with water from the original culture. The tank was covered with black cloth on the bottom and all sides. A removable lid was put on top to prevent light penetration from outside (Fig. 2-2). Ten individuals of *E. braueriana* were introduced in the tank each time. After ten minutes for acclimation, light from compact fluorescent lamps was introduced through a round hole at one corner of the lid. Four separate illumination treatments were conducted: 14 W, 18 W, 23 W or 27 W (Philips E27). The beam of light was perpendicular to the water surface. Table 1 shows the luminous flux per Watt (lumen/W) given by the product producers and intensity (lux) measured with a TES-1339 light meter set up in the testing tank. After a five minute illumination period, which proved to be sufficient for the animals to cross the tank length in preliminary tests, the position and number of individuals were recorded, with the ratio of individuals on the bright side to the total number of individuals in the tank stated as *R,* representing their phototactic tendency. If the ratio is significantly higher than 0.5, it represents positive phototaxis; whereas if *R* is significantly lower than 0.5, negative phototaxis is indicated. If the ratio is not significantly different from 0.5, it is regarded as showing no phototaxis. In total, each of the three developmental stages was exposed separately to the four illuminance treatments, resulting in a total of twelve separate treatments. Each treatment was replicated ten times.

Analysis

All data were analyzed with software R 2.15.2. and R 3.0.3 (R core team, 2014). The Kolmogorov-Smirnov test was used to determine phototactic tendency in each treatment. ANOVA and Tukey's HSD test were used to analyze responses between treatments. The data were transformed following the Aligned Rank Transform (ART) procedure of Wobbrock *et al.* (2011) beforehand because normal assumptions were not met. Interaction of the two variables was tested with ART using the package ARTool (Kay and Wobbrock, 2014).

Results

The results demonstrate that phototaxis of *E. braueriana* varied with both age and light intensity (Fig. 2-3). The main effects of developmental stage (ANOVA df = 2, F = 112.82, $p < 0.01$) and illuminance (ANOVA df = 3, F = 21.56, $p < 0.01$) were both significant as well as the interaction effect (ANOVA df=1, F = 10.10, $p < 0.01$). Tukey's HSD test indicated that the mean R of the nauplii (0.82±0.09) were significantly different from those of the juveniles (0.28±0.24) and adults (0.25±0.25). Considering illuminance, the R values under 14 W light (0.66±0.22) were significantly different from that of 18 W (0.40±0.33), 23 W (0.41±0.33) and 27 W (0.34±0.36).

The R values of the nauplii were significantly higher than 0.5 in all four treatments (Kolmogorov-Smirnov test $p < 0.01$ for all treatments). Responses were not significantly different between the treatments (ANOVA df=3, F = 0.25, p = 0.86) indicating consistent positive phototaxis uninfluenced by illuminance.

In juveniles and adults, R was significantly lower than 0.5 (Kolmogorov-Smirnov test $p < 0.01$ for 18 W, 23 W and 27 W in both the juvenile and adult stage) except in the 14 W treatment ($p = 0.15$ and $p = 0.16$ for juveniles and adults, respectively). These results indicate that both juveniles and adults showed significant and consistent negative phototaxis when the illumination was higher than the 14 W treatment.

Discussion

Our results demonstrate that phototactic response of *E. braueriana* differed with both developmental stage and illuminance. The nauplii stayed positively oriented toward light under all illuminance treatments. But the juveniles and adults showed negative phototaxis in all except the lowest illuminance (14 W), where they showed no response. In general, *E. braueriana* showed reversing phototaxis which could be associated with light reception, foraging, distribution and dispersal.

Nauplii of *E. braueriana* are able to react to light in that they possess a region of photoreceptors called the frontal eyes, including the four-partite nauplius eye and the frontal organs (Meyer-Rochow, 2001; Elofsson, 2006). Composed of pigment cells, rhabdomes and inverse sensory cells, the frontal eye is able to perceive light and wavelengths while in most species regarded as unable to create visual images (Walker et al., 1987; Criel, 1991). Studies have shown that the removal of the frontal eyes sometimes affects light induced vertical locomotion and UV perception (Waterman, 1961). The frontal eyes are present in *E. braueriana* at hatching, enabling simple orientation. Positive phototaxis in the nauplius stage drives them away from the bottom, moving upwards to stay near the water surface during daylight. Such movement could benefit the animal in feeding and dispersal. The branchiopod nauplius is generally regarded to hatch lecithotrophic (Fryer, 1983; Fryer, 1988; Eder, 2002), maybe becoming facultative lecithotrophic with time and then eventually turning to filter feeding (Olesen and Grygier, 2003; Fryer, 1983; Cannon, 1928) before gradually acquiring the adult feeding mode during the juvenile stage. Allen and Pernet (2007) hypothesized that this process might be an adaptation for food limitation at the hatching site and the subsequent dispersal away from it. In seasonally astatic wetlands, benthic particles are generally agitated during inundation but planktonic organisms may not be numerous so early in the hydroperiod. While nauplii in wetlands do not travel long distances as do marine larvae, lecithotrophy still allows them to bridge time instead of distance. As planktonic food source increase with time, positive phototaxis would be beneficial for feeding on pelagic particles (Spooner, 1933). Furthermore, as they hatch from the bottom in large numbers within a short time period, swimming to the surface could also alleviate density pressure near the benthic region and reduce intraspecific competition (Coutant, 1982). Thus, lecithotrophy combined with positive phototaxis may play an important role in the early survival of these animals.

Juvenile and adults are in many aspects different from the nauplius stage. The development from nauplius to juvenile sees a significant change in their phototaxis, suggesting a transformation in living strategy. From the juvenile stage on, *E. braueriana* now have compound eyes for visual perception, movable thoracopods for food gathering and a bivalve carapace. They stayed mostly near the bottom of the water (personal observation), which could be related to negative phototaxis. Adults have also often been observed to gather on unvegetated surfaces in Siangtian Pond, such as immersed rocks and human walked paths (Chou, 2008). Their feeding structure morphology indicates that juvenile and adult *E. braueriana* are filter feeders but may also scrape on periphyton (in preparation). Combined with their phototactic tendency, we suggest that *E. braueriana* is a benthic feeder. Negative phototaxis thus keeps them near their food source.

Furthermore, *E. braueriana* is unable to swim during reproduction, no matter if it is self-fertilization or sexual copulation (personal observation), much like *Eulimnadia texana* (Packard, 1871) (Knoll, 1995). In some aquatic species, positive phototaxis benefits in finding mates visually. In *E. braueriana*, however, the majority of the population consists of hermaphrodites, which may reduce the need for outcrossing. Moreover, if males rely on tactile cues or chemical signals rather than vision to find receptive hermaphrodites, as proposed for *E. texana* (Weeks and Benvenuto, 2008), there would be even less need for positive phototaxis.

After hatching from the bottom and gathering near the surface with positive phototaxis at the nauplius stage, this adverse phototactic tendency will eventually guide the juveniles and adults back deep into the water column on bright days. This bottom-surface-bottom distribution is similar to those of sessile Crustaceans with a pelagic larval stage, such as barnacles. Studies on such species have suggested that the change in phototaxis is highly beneficial and also important for the sustaining of those species. Even if being pelagic does not bring trophic advantages, the advantage of dispersal and colonizing diverse environments alone would be highly beneficial (Thorson, 1964; Crisp, 1976). In the case of barnacles (Thorson, 1964; Barnes and Klepal, 1972), pelagic larva have positive phototaxis which helps them reach the water surface. Once arrived, they are able to disperse wide distances with the help of surface currents to find settling locations. To settle down, however, phototaxis changes from positive to negative. It is possible that during the similar cycle of distribution, *E. braueriana* disperse away from their hatching place not only vertically but also horizontally, thus distributing wider or more evenly in their habitat. Though they cannot benefit from large scale dispersal, Siangtian Pond’s diverse topography still harbors different microhabitats that could influence the timing and condition concerning the hatching of their offspring (Wang *et al.*, 2014). Wider distribution thus increases the sustainability of *E. braueriana* in a stochastic environment.

Phototaxis, however, should not be the only factor affecting distribution in the juvenile and adult stages. From our results we also know that juveniles and adults show varying response under different illuminance. Although mostly being photonegative, light only affects their distribution above certain levels. This suggests that distribution is indeed limited by light but it should be influenced by other factors under dim conditions, such as food preference or the limitation of food handling ability. No reaction to low illuminance may also imply that the juvenile and adult *E. braueriana* would less likely exhibit diel vertical migration, in which the animal would be attracted to dim light. Diel vertical migration is mostly associated with avoidance of visual predators in the water column (Forward, 1988; Forward and Hettler, 1992; De Robertis et al., 2000). Several large branchiopods are known to perform cannibalism on nauplii or smaller individuals, leading to nutritional benefits or manipulation of population density (Dumont and Ali, 2004; Lakka, 2015). Davis and Madison (2000) suggested that in the case of *Triops longicaudatus* (LeConte, 1846), ontogenic phototaxis shift creates vertical segregation between the nauplii and adults, thus reducing unintentional cannibalism. *E. braueriana* shows identical phototaxis shift, potentially avoiding cannibalism, but overlapping cohorts have not been observed both in the wild and in laboratory cultivation (personal observation). In Siangtian Pond, some predatory insects, including diving beetles, backswimmers and dragonfly nymphs, colonize when the hydroperiod is long enough. It is a general case that predators arrive later than branchiopods in a temporary pond (Macan, 1963; Jocque *et al.*, 2010). The nauplii that hatch early might have lower risk of encountering predators even when they show positive phototaxis and gather at bright areas. Their small size and transparent body could also be beneficial in this case, whereas the adults are not only much bigger in size but also carry whitish eggs that could be more visible to predators (De Robertis et al., 2000). Juveniles and adults possess a soft carapace that might be able to provide slight protection. But in the short-lived temporary ponds, quick development and reproduction are among the first priorities (Hildrew, 1985; Brendonck *et al.*, 2000). Whether the predators assert sufficient pressure on the population to induce changes in behavior or morphology (Brendonck *et al.*, 2002; Rabus *et al.*, 2012) is not clear yet.

Siangtian pond is also habitat of *Branchinella kugenumaensis* and *Lynceus biformis*. In the wild, vertical distribution of *B. kugenumaensis* and *L. biformis* were significantly different, gathering at the surface and at the bottom respectively (Wang et al., 2012). *E. braueriana* showed a tendency to gather near the bottom but there was no statistical significance, possibly due to low population size (Wang et al., 2012). From the negative phototaxis found in this study, we suggest that *E. braueriana* has benthic preference similar to *L. biformis*, different from the pelagic *B. kugenumaensis*. But despite similar vertical distribution, *E. braueriana* and *L. biformis* have varying horizontal hotspots, possibly indicating other ecological differentiations between the two species. Differentiation in resource utilization is advantageous in such a highly synchronized and short-lived community (Wang *et al.*, 2012). Aside from spatial difference, foraging ability could be among the main factors in resource partitioning.

In conclusion, *Eulimnadia braueriana* changed its phototactic tendency through both development and illuminance. Nauplii showed consistent positive phototaxis while juveniles and adults were negatively oriented to light except of showing no response to the lowest illuminance. Such change would facilitate vertical migration from the bottom to surface and back to the bottom during their development. This migration could pose benefits in avoiding intraspecific competition, reducing excessive waste of energy, staying near to food source, and distributing wider throughout the habitat. We suggest that *E. braueriana* changes its living strategy at least once during its life cycle, between the nauplius and juvenile stage, and achieve thereby better sustainability in a stochastic temporary pond. This study provides basic information that will be helpful in further ontogenic studies. Deeper understanding on locomotion and feeding ability could further determine the cause and effects of changing phototaxis, illustrating a broader picture of *E. braueriana* living strategies.

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Table 1. Luminous flux per Watt and measured illuminance of the compact fluorescent light bulbs used in this study.

	14W	18W	23W	27W
lumen/W	54	57	61	62
lx	19,688±127.6	21,948±272.8	38,266±319.1	48,286±229.5

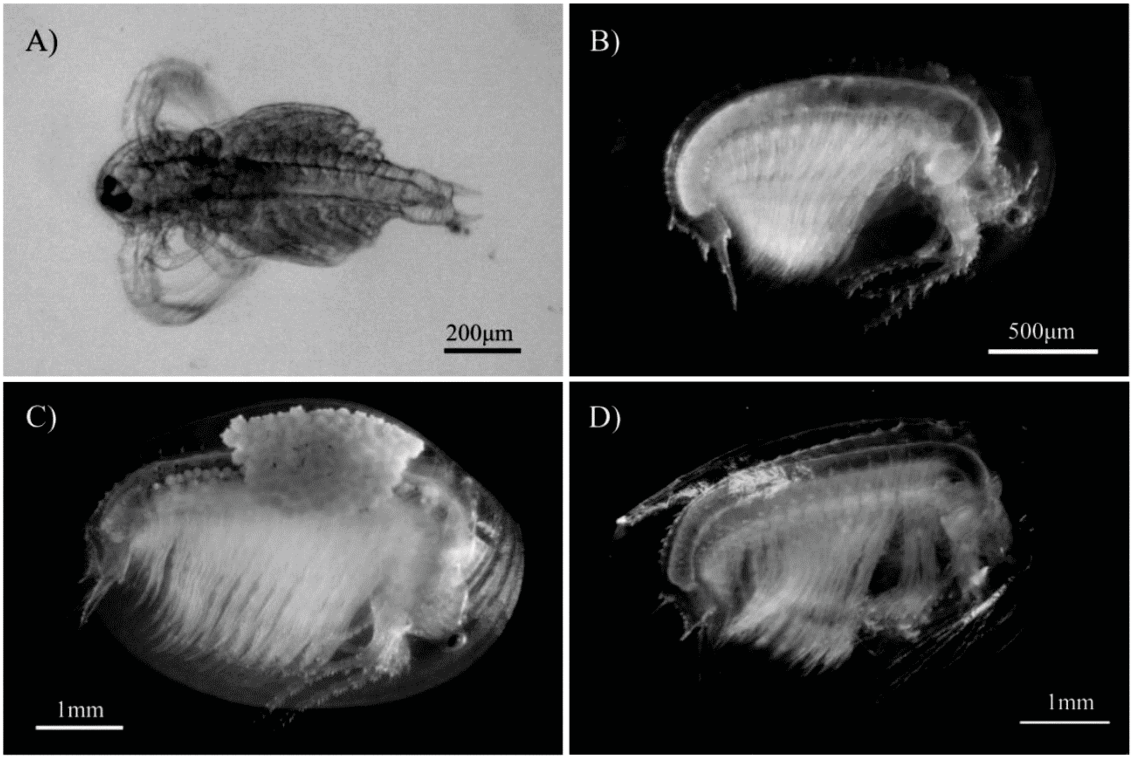

Figure 2-1. *Eulimnadia braueriana.* a) Nauplius stage. Thoracopods are still unable of locomotion and without carapace. b) Juvenile stage. The carapace is fully developed and all appendages are movable. c) Adult stage hermaphrodite. Eggs are synthesized along the gut and carried on its dorsal side. d) Adult stage male. The first two pairs of thoracopods are specialized as clasper.

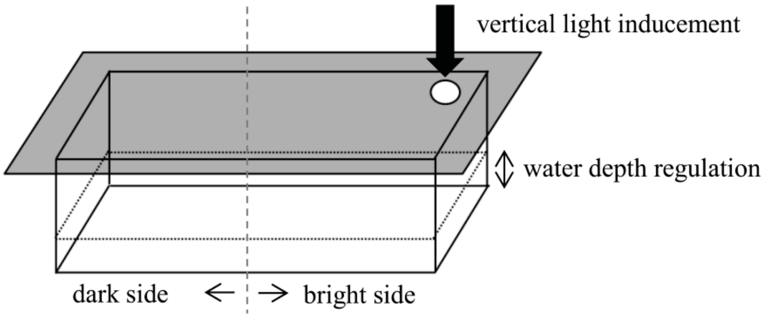

Figure 2-2. Experimental set-up for testing ontogenic phototaxis in *Eulimnadia braueriana* across illuminance (see text for detail).

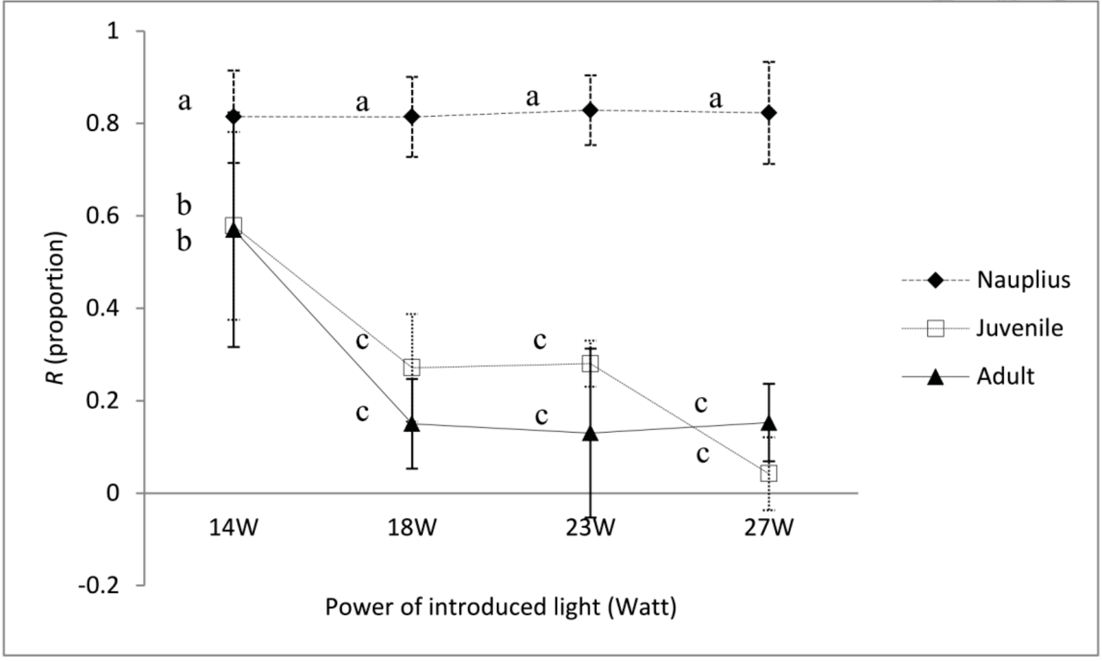

Figure 2-3. Phototactic tendency of *Eulimnadia braueriana* in each developmental stage through the four treatments. Lowercase letters denote the groupings after Tukey's HSD test for both treatments and developmental stages where it was significantly different between groups (bars represent standard deviation of the mean).

Chapter 3: Developmental and functional morphology of *Eulimnadia braueriana* Ishikawa, 1895 feeding structures with comparison of adult sympatric large Branchiopoda (Crustacea)

Jer-Yuh Liu, Chun-Chieh Wang, D. Christopher Rogers, Lien-Siang Chou

Abstract

Branchiopod functional morphology has mostly been studied either in adults or in larvae, seldom has it been compared collectively. We examined the feeding structures in *Eulimnadia braueriana* from nauplius to adult, to clarify feeding mechanisms. This species lives sympatrically with two other large branchiopods in Yangmingshan National Park, Taiwan. Naupliar feeding structures were similar to those of other studied species, suggesting filter feeding. The naupliar feeding structures, including the mandiblular palp and the naupliar process, gradually degenerate during the juvenile stage. Simultaneously, the molar surface, maxillae and second antenna develop, reaching their adult form in the later juvenile stages. The molar surface and thoracopod setae morphology are similar to those of other filter feeding animals. But adults also have scraping setae on the first several pairs of thoracopods. Nearly all naupliar primary feeding structures change during development, whereas late juvenile and adult morphology are similar. This suggests that most significant morphology and feeding changes occur in the early juvenile stages. Comparison between molar and thoracopod morphology with sympatric large branchiopod taxa revealed potential competition and resource partitioning among species.

Keywords: *Branchinella kugenumaensis*, *Lynceus biformis,* SEM

Introduction

Crustaceans have diverse morphologies and life strategies, allowing them to adapt to a wide variety of environments. Crustacean morphological studies have elucidated taxonomic evolutionary histories (Fryer, 1985; Lindholm, 2014) and diversification (Watling, 1989; Mura, 1996; Olesen, 2007). The linkage of morphology and function also demonstrates behavioral strategies, such as feeding (Storch, 1925; Ghauri, 1983) and reproduction (Plodsomboon *et al.*, 2012; Sigvardt and Olesen, 2014). Large branchiopod feeding modes have been reported as four primary types: predatory, scavenging, filter-feeding and scraping.

Filter feeding is probably the original and most common feeding mode in branchiopods (Cannon and Leak, 1932) and is characterized by dense, plumose setae on the thoracopods which create a feeding current as the thoracopods move in metachronal synchronicity (Cannon, 1932; Fryer, 1983). Such dense setation creates a tight mesh that captures and transfers particles along the food groove (Cannon, 1932; Fryer, 1983).

Predatory morphological indicators are sharp, robust and larger thoracopod spines and molar teeth (Fryer, 1966; Martin and Cash-Clark, 1995; Rogers et al., 2006). These characters can be found in certain Cladocera (Martin and Cash-Clark, 1995), Anostraca (Fryer, 1983; Rogers et al., 2006) and Notostraca (Fryer, 1988).

Although morphological traits of scraping or scavenging species are similar to filter feeders, they generally have stout setae or spines on the thoracopod portions that come in contact with the substrate (Martin, 1989; Fryer and Boxshall, 2009). Their mouth parts may also vary slightly in that they can ingest harder particles than filter feeders (Ghauri, 1983; Richter, 2004; Fryer and Boxshall, 2009). There are also species in which the feeding structures have multiple functions, keeping a more complex diet under different circumstances (Mertens *et al.*, 1990; Brendonck, 1993).

Spinicaudatan branchiopod feeding modes vary greatly. The related Laevicaudata have been described as scrapers (Martin, 1989; Fryer and Boxshall, 2009) while the thoracopods and carapace generate a steady water flow along the food groove, drawing the scraped particles towards the mouth (Cannon, 1932; Fryer, 1983). In Spinicaudata, scraping structures were identified in *Imnadia* (Botnariuc, 1947); filter feeding was suggested in *Limnadia*, *Cyzicus* and *Leptestheria* (Storch, 1925; Cannon, 1932; Emberton, 1980; Martin and Cash-Clark, 1995). Although these few studies cover all spinicaudatan families, information on feeding mode development is generally lacking. Information on branchiopod nauplii has been important in taxonomic and evolutionary studies (Pai, 1958; Williams, 1994; Scholtz, 2004; Fritsch and Richter, 2012). And for species where the nauplius and adult have widely different morphology or living habits, as in *Eulimnadia braueriana* Ishikawa, 1895, the juvenile stage is an intermediate period with prominent changes. Thus, ontogenic development studies may bridge the gap between nauplius and adult behavior.

Eulimnadia braueriana in Taiwan is found sympatric with two other large branchiopod species, the anostracan *Branchinella kugenumaensis* (Ishikawa, 1895) and laevicaudatan *Lynceus biformis* (Ishikawa, 1895), in Siangtian Pond, Taipei. Siangtian Pond has inundation periods that often last shorter than the optimal branchiopod life spans due to porous bottom substrate (Huang et al., 2010; Wang et al., 2012). The bowl shaped basin causes the submerged area to decrease with depth, so crowding near the end of a hydroperiod is very common (Wang et al., 2012). Previous studies demonstrate partial vertical segregation among the Siangtian Pond large branchiopod species (Wang et al, 2012). Furthermore, *E. braueriana* displayed a change in phototactic response (from positive to negative) between the nauplius and juvenile stage (Liu et al., under review) demonstrating vertical segregation within the species, which may be related to foraging.

We examined *E. braueriana* feeding structure morphology across developmental ontogeny and then compared the primary feeding structures with the co-occurring large branchiopod taxa. By comparing and contrasting the primary feeding structures of these taxa we can determine their feeding patterns and look for evidence of resource partitioning.

Material and Methods

Eulimnadia braueriana specimens were cultured in the laboratory with aged tap water and 12/12 hours light at 22-24°C or sampled from the field during the 2014 hydroperiod (late May to mid-June). Specimens of *Lynceus biformis* and *Branchinella kugenumaensis* were obtained during the same hydroperiod. Fresh specimens were kept on ice for a short period to cease movement before they were preserved in 5% formalin overnight at 4°C and then were serially dehydrated to 70% ethanol for storage.

General observation and dissection were made using a Leica mz125 light microscope. Photographs were taken with Canon EOS 550D mounted on the light microscope with an adapter. Samples prepared for scanning electron microscopy (SEM) were serial dehydrated to 100% ethanol and changed to 100% acetone before critical point drying (Appendix 1). They were then mounted on carbon tape and coated with gold and then observed with FEI Inspect S SEM and Hitachi Tabletop TM-3000 SEM.

Developmental stages were determined based on morphology. Nauplii are defined as individuals without a complete carapace. This form was further divided into seven substages following Olesen and Grygier (2003). Juveniles are defined as possessing a fully grown carapace but have not reached sexual maturity. Based on the developed number of setose thoracopods, the juvenile stage was divided into the early (fewer than ten setose thoracopods) and late (more than ten setose thoracopods) juvenile stages. Adults have a fully developed carapace and mature reproductive structures. Males, which are relatively rare in this species (Huang and Chou, 2015), can be identified by the first two thoracopod pairs that are modified as claspers. Hermaphrodites are characterized by the whitish eggs that are visible in the ovaries or on the dorsal side of the body.

Results

General morphology

Eulimnadia braueriana shows gradual morphological transformation until reaching maturity. The first nauplius substage has an oval trunk which elongates with development, and a large pair of second antennae (Fig. 1 (A)). The labrum is triangular and covers most of the ventral trunk surface. The telson becomes more pointed (Fig. 3-2(A)) and the carapace anlage starts to form at nauplius substage five (Fig. 1 (E)). At substage seven the animal curves ventrally (Fig. 1 (G)). Thoracopod buds are visible in this and the previous substage, but they are still underdeveloped and unable to move. Upon reaching the first juvenile substage, the carapace covers the trunk and all thoracopods. Growth lines on the carapace are not seen in the juvenile stage and the number of setose thoracopods develops from seven to eleven pairs before maturation. The telson gradually develops during these substages (Fig. 1 (H) – (I)). After maturation, individuals bear ova or have two pairs of claspers (Fig. 1 (J) – (L)). In large hermaphrodites, the carapace dorsal margin is slightly arcuate, creating more space to carry eggs.

Naupliar second antenna

The second antenna is the most prominent appendage in the nauplius stage. It begins to move upon hatching and can be divided into three antennomeres: the protopod, endopod and exopod (Fig. 3-2 (A), Fig. 3-3 (A)).

The protopod is generally smooth. The basal portion is the protopodal coxa, which bears the elongated, conical naupliar process (or proximal masticatory spine) that becomes bifid at the fourth substage. The distal masticatory spine is located distoposteriorly on the protopod, bearing well-spaced setae in the distal half. At the protopod apex project the anterior endopod and the posterior exopod.

The endopod is slightly shorter than the exopod (Fig. 3-3 (A)). The exopod bears a subdistal, posterioventral row of five articulated setae each with a single row of short setules from approximately mid-length to the distal apex. The endopod setae are concentrated at the distal margin, each with a distal row of short setules.

The distal masticatory spine and the naupliar process on the protopod are mildly curved medially (Fig. 3-2 (A)). It is apically bifid from the fourth substage on. The setules on the posterior branch are unevenly arranged whereas those of the anterior branch are two parallel, longitudinal rows with apices directed distally (Fig. 3-3 (C)). During the anterior-posterior strokes of the second antenna, the naupliar process reachs below the labrum on the posterior stroke (Fig. 3-2 (A)). The distal masticatory spine has sparse, slender setae from the second naupliar substage on (Fig. 3-3 (A), the third substage).

Nauplius mandibles and maxillae

The mandible is the only other movable appendage in the nauplius stage, with a similar movement pattern as the second antenna. It can be separated into the coxa and mandibular palp (Fig. 3-2 (A), Fig. 3-4 (A), (B)).

The coxa starts as a bud at the first substage (Fig. 3-4 (C)) but rapidly develops medially where the two molar surfaces finally touch at approximately the fourth substage (Fig. 3-4 (D), (E)). The molar surface is long and narrow and the spines are still simple. The general morphology remains similar during the nauplius stage, except the first substage. The molar surface has anterior cusps, a posterior dentiform structure and several rows of ornamented setae even before the left and right counterparts meet at midline. The distal arc of the coxa supports the mandibular palp, which projects at a right angle from the coxa.

The mandibular palp is approximately the same length as the coxa and consists of three palpomeres, each bearing two spines (Fig. 3-4 (B)). The spines on the first palpomere reach beneath the labrum during a posterior stroke. After the first naupliar substage, both spines on the first palpomere bear distal tufts of plumose setae. The proximal spine setae are unevenly distributed on the anterior surface and are directed to the mouth, similarly to the anterior tip of the naupliar process. The distal spine setae are arranged circularly around half the apex (Fig. 3-4 (B)).

The naupliar maxillae are small setae in the first substage (Fig. 3-4 (A), (C)). Maxilla buds appears at substage six. By the last substage, the first maxillae have a slight beginning food groove between them, with sparse setae vaguely directed to the mouth (Fig. 3-4 (F)). The second maxillae are only visible in the last two substages as buds without setation (Fig. 3-4 (F)).

Juvenile and adult second antenna

The second antenna of the adult consists of the indistinctly segmented protopod, endopod and exopod (Fig. 2 (C), Fig. 3-5 (E), (G)).

The naupliar process and distal masticatory spine on the second antenna slowly degenerate and disappear across the juvenile stages (Fig. 3-5 (A), (B), (C), Fig. 3-6 (A)). The endopod and exopod are annulated, with two setae on each antennomere except the distal most one which has three setae, and elongate as the masticatory spines degenerate, matching the length of the protopod at the juvenile stage (Fig. 3-5 (C)). The number of antennomeres increases with development, reaching seven on the endopod and eight on the exopod at maturation (Fig. 3-5 (E)). There protopod anterior surface bears longitudinal rows of plumose setae (Fig. 3-5 (G)).

During the early juvenile stages, the endopod and exopod bear long, distal setae (Fig. 3-5 (A)). At the later juvenile stages, each antennomere bears one short and one long seta on the anterior and posterior surface, respectively (Fig. 3-5 (C)). Eventually, four to five long setae are distributed along the posterior surface of each adult antennomere (Fig. 3-5 (E), (F)).

Juvenile and adult mandibles and maxillae

In *E. braueriana*, the mandibular palp degenerates through the juvenile stages and is quickly outgrown by the coxa (Fig. 3-6 (A)). The mandibular palp spines reduce and are no longer able to reach near the mouth, eventually disappearing before the animal reachs maturity.

The molar surface widens in the juvenile stage and the spines start to differentiate morphologically (Fig. 3-6 (B)). The molars quickly become asymmetric. On the right molar surface, the cusps at the anterior region become broader at the border and both flagellated and simple processes are seen in the posterior region. The ventral border of the left molar surface consists of flagellated processes, with a particularly large process at the anterior end. Distinct from the naupliar molar surface, the setae that had been present near the posterior tooth decrease in number (Fig. 3-6 (B)).

In the adults, the molar surface is differentiated (Fig. 3-7, Fig. 3-8). Area definitions follow the descriptions of *Streptocephalus coomansi* Brendonck and Belk, 1993 (Brendonck, 1994). The posteriodorsal area is relatively small and bears only one single large tooth (Fig. 3-8 (F)). It separates the peripheral region and the ‘region with bordering flagellated processes’. The peripheral region is narrow and has simple processes (Fig. 3-8 (E)). The border on the other side show flagellated processes (Fig. 3-8 (B), (C)). Processes on the anterior end are broad and flat while those on the posterior end are slender (Fig. 3-8 (D)). The transitional zone starts next to the posterior end. Here the flagellated processes are substituted by blunt protrusions. The central region constitutes the largest area on the molar surface; it is smooth and perforated (Fig. 3-8 (A)).

The first maxillae are fully developed in the juvenile stage, equipped with long plumose setae that reach to the mandible coxa (Fig. 3-9). Setal morphology (Fig. 3-9 (C)) strongly resembles the plumose thoracic endites (Fig. 3-10 (E)). The second maxillae are flat and also bear dense setae that are directed anteriomedially (Fig. 3-9 (A)). They are approximately half as large as the first maxillae. Lateral to the second maxillae are the labral gland openings (Fig. 3-9 (A)).

The mandibles of *Branchinella kugenumaensis* also have molar surfaces with obvious morphological distinction in different areas (Fig. 3-7 (B), Fig. 3-10 (A)) like most other non-predatory Anostracans. At the dorso-posterior surface, where *E. braueriana* has a posterior tooth, are a row of flagellated, conical teeth (Fig. 3-10 (C)). The teeth decrease in size to the anterior margin and decrease both in size and complexity posteriorly until they are simple cusps on the posterior most margin and slender, flagellated spines at the dorso-anterior border (or ‘the region of bordering flagellated process’). Several small protrusions are present at the transition area between the dorso-posterior border and the central region (Fig. 3-10 (C)). The peripheral region consists of spines with multiple flagellations. They decrease in diameter and complexity anteriorly. Immediately medial to the thin anterior spines are several rows of teeth that are rectangular in distal viewe and bear a variety of conical cusps (Fig. 3-10 (C)). Near the central region, these cusps are less developed and are arranged in closer proximity to each other. The central region is broad, smooth and perforated just like in *E. braueriana*.

Conversly, *Lynceus biformis* has the characteristic laevicaudatan molar surface (Martin, 1989; Richter, 2004). It is long and narrow, with 11 dorsoventral ridges and grooves (Fig. 3-11 (A), (B)). The ridges are crescent shaped with higher spines on the two ends and lower spines in the middle.

Juvenile and adult thoracopods

Juvenile *E. braueriana* has seven pairs of movable thoracopods in the first substage, decreasing in size and maturity posteriorly (Fig. 3-2 (C)). A fully developed thoracopod consists of five medial endites (Fig. 3-12 (A)), a distal endopod (Fig. 3-10 (C)), a basolateral epipod and a distolateral exopod (Fig. 3-12 (B)). The first two thoracopods pairs in adult males are specialized as claspers (Fig. 1 (L)), whereas in hermaphrodites, the ninth and tenth thoracopods pairs have dorsally extended exopodal filaments for egg attachment. In unspecialized thoracopods, the epipod is the only lobe without marginal setae (Fig. 3-12 (B)). The exopod has a dorsal and a ventral extension. Both bear loosely distributed marginal setae. The endites and the endopod are used in feeding. They have dense, plumose marginal setae (Fig. 3-12 (D), (E)) forming a mesh with the setae of the adjacent thoracopods. Endite I, also called the proximal endite (Pabst and Richter, 2004) is slightly elongated and subacute (Fig. 3-12 (A), (C)). It bears dense plumose setae on the ventral side, two slightly thicker setae on the distal side, and two short, stout setae with a row of triangular teeth on the posterior side (Fig. 3-12 (C)). The remaining endites all have two parallel rows of dense, plumose setae (Fig. 3-12 (D)). There is a palp on endite V of the first six thoracopod pairs, bearing long, robust, pectenate distal setae on the distal end (Fig. 3-12 (E)). These setae are similar to the pectenate structures found on laevicaudatan thoracopods.

The eleven pairs of *B. kugenumaensis* thoracopods are biramous, with six medial endites, a distal endopod, a lateral exopod and the basolateral praepipod and epipod (Fig. 3-14 (A)). The setae on the lateral lobes are sparse compared to the long, dense and plumose setae of the medial endites (Fig. 3-13 (B)). The first three endites are broad, whereas the distal three are much smaller and bear setae with shorter setules (Fig. 3-13

(C)). The endite setation is similar to that of *E. braueriana*.

Lynceus biformis thoracopods bear dense setae on both the medial and lateral lobes (Fig. 3-13 (A)). There are five medial endites, a distal endopod, a basolateral epipod and a distolateral exopod with a dorsal extension. In males, the first thoracopod pair is specialized as claspers; in females, the eighth and ninth thoracopods are specialized for egg attachment. The thoracopods diminish in size posteriorly. On the distal end of the endopod and endite IV and V, *L. biformis* possess robust pectenate setae (Fig. 3-13 (B)). The thoracopods of *L. biformis* are similar to those of *E. braueriana* but have notably more robust pectinate setae.

Discussion

Morphology is reflective of function, making it a study choice when functions are not easily observed, such as the feeding process of the ventrally bent clam shrimps. Gut content analysis is another often used study method, but it only reveals what had been ingested during a certain time period in that one environment. Feeding morphology shows the overall ability of food handling and ingesting, results are also less influenced by fluctuating environment. From feeding structures we can know the feeding method of a species and what it is able to ingest in any given environment. From *E. braueriana* feeding structure morphology we found that their feeding method changes with development. Morphology also revealed the potential feeding relation of the three sympatric species.

Eulimnadia braueriana experiences several different feeding mechanisms through its development. At the first naupliar substage, yolk is visibly embodied in the trunk. The whole trunk appears yellowish, so dense that the gut is not visible. Moreover, the mandible coxa are only buds, indicating that this substage is exclusively lecithotrophic. Yolk is gradually consumed but will persist through several successive naupliar substages. The two molar surfaces are able to meet at approximately the fourth substage, which was also observed in the same species (Olesen and Grygier, 2003) and in

Limnadia stanleyana King, 1855 (Anderson, 1967). They could be exclusive lecithotrophic until the third substage, relying entirely on yolk nutrition. Traces of yolk, however, can sometimes still be seen in substage five. There may be a transition period in which the nauplius is partially lecithotrophic before being able to forage independently. Following this assumption, only the last two nauplius substages are totally planktotrophic.

The main naupliar structures that gather food particles to the mouth are the second antenna masticatory spines and the mandibular palp spines. This system is reported to filter planktonic particles (Scholtz, 2004). The appendage setae possess setules that filter particles from the water. The second antennal posterior stroke not only facilitates locomotion but also gathers particles along its path and pushes those particles into the mouth.

But this system degenerates during the juvenile stage. With the degeneration of the naupliar process and distal masticatory spine, the second antenna loses its feeding function. The spinicaudatan second antenna is the only structure responsible for locomotion (Cannon, 1932; Olesen, 2009). The developed setose, movable thoracopods and the maxillae take over the feeding role upon entering the juvenile stage. In general, the anterior cephalic food handling structures are substituted by posterior cephalic and thoracic structures in the juvenile stage. This posterior shift was also observed in other arthropods (Averof and Patel, 1997; Møller et al., 2007).

Eulimnadia braueriana juveniles and adults share similar morphology. At the early juvenile substages, the molar surface is less specialized and they only possess half as many thoracopods as the adult stage. *E. braueriana* endites possess two parallel rows of dense, plumose setae, as has been observed in other filter feeders (Cannon, 1932). Such dense setae form a "wall" of the food groove, enabling it to create an internal water current that helps transport food particles. The endite palps also bear pectinate setae that are similar to the scraping setae of *Lynceus* (Martin, 1989; Fryer and Boxshall, 2009). As these palpal setae are also the distal most limb structure, able to touch external surfaces, we suspect they have scraping function as in *Lynceus*. Botnariuc (1947) mentions such scraping setae in the spinicaudatan *Imnadia* and there are some studies that also found similar setae in other spinicaudatan genera (Straskraba, 1965; Roessler, 1991).

The difference of the setae between *E. braueriana* and *Lynceus* are the location and abundance. In *E. braueriana*, they are only present on the endite V palp. In *Lynceus*, however, they are present on the two distal-most endites and the endopod (Fryer and Boxshall, 2009). The *Lynceus* scraping setae outnumber those of *E. braueriana*. Since scraping setae have less dense setules, they are not as efficient in filtering as plumose setae. The arrangement of setae indicate that the *Lynceus* are less filtratory than *E.*

braueriana but are compensated with better scraping ability. It appears that *E. braueriana* has limited scraping abilities and relies more on filtering.

Scraped or filtered particles are transported proximally into the food groove by the endites. Inside the food groove, particles are either transported by the water current or mechanically pushed anteriorly with the beating of the thoracopods (Martin, 1989). The stout, pointed endite I may function in pushing larger particles forward (Cannon, 1932). The gap between the anterior most thoracopods and the mandibles is closed by the first and second maxillae. It has been suggested that the food particles transported beyond the thoracopods easily scatter outwards of the food groove before they reach the mandibles. The labral gland opening lateral to the second maxillae may produce a secretion to entangle the food particles into a mass for easier transportation (Zeni and Zaffagnini, 1992). The first maxillae have setae with morphology very similar to the dense, plumose setae of the endites. They should have a similar filtering and particle-transporting function (Fryer, 1983; Fryer and Boxshall, 2009).

The food mass must then pass the molar surface from posterior to anterior. The large tooth on the mandible posteriodorsal side has been suggested to be homologous with the *pars incisivus*, which usually has biting functions (Richter 2004; Edgecombe et al., 2003). It could be able to crush larger particles before they reach the finer structures further anterior on the molar surface (Edgecombe et al., 2003). The *E.braueriana* molar surface has similar structure as some filter-feeding anostracans (Brendonck, 1994; Mura, 1996). Nevertheless it is debatable whether the molar surface form is informative of clam shrimp feeding mechanisms (Mura, 1996).

Eulimnadia braueriana initially adopts planktonic filtering and then shifts to filtering with some scraping. Although they possess filtering ability as long as they are able to feed independently, the nauplii should filter different resources than the juveniles and adults. In addition to the difference in body size and thus the accessible size of food particles, the feeding structures become more diverse. The fully developed mandible, maxillae and the thoracopods with dense, plumose and pectanate setae can utilize a wider food variety, including planktonic and sessile materials. Moreover, since the naupii showed opposite phototaxis compared with the juveniles and adults, leading to habitat partitioning (Liu *et al.*, under review), they probably feed on distinct resources in different microhabitats.

Adult *Lynceus biformis* and *E. braueriana* may share similar vertical distribution (Wang et al., 2012; Liu et al., under review). The pectinate setae on the endites and exopod also indicate similar scraping function, revealing possible competition. Their molar surface, however, show that they should have some degree of resource partitioning. There is a distinct border of the molar surface composed of flagellated or simple processes in *E. braueriana* but the largest area is the smooth, perforated central region. The molar surface of *L biformis* does not show any wide, smooth area. Neither does it have slender processes. It is uniformly constituted of rough dorsoventral ridges. Another difference is that the *Lynceus'* left and right molar surfaces are symmetric, which implies that no biting is involved, only grinding (Richter, 2004).

Branchinella kugenumaensis share similar dense, plumose endite setae with *E. braueriana* but not the scraping setae. Molar surface morphology is also alike. Their filtering ability might be similar but they show different preference for vertical distribution (Wang et al., 2012). Combining distribution and information of feeding structures, we suggest that *E. braueriana, L. biformis* and *B. kugenumaensis* feed on different resources. But when aquatic space is too limited for spatial segregation, *E. braueriana* might face competition from both *B. kugenumaensis* and *L. biformis* since it has similarities with either one, while *B. kugenumaensis* and *L. biformis* still maintain some resource partitioning.

In conclusion, we focused on the morphological development of feeding structures in *E. braueriana* and made a preliminary comparison among adult feeding structures of all three large branchiopod species inhabiting Siangtian pond. Morphology of *E. braueriana* developed gradually, with the most prominent changes happening during the early juvenile instars. In adults, we found scraping structures similar to those of laevicaudatans. From structure and their previous studied phototaxis, we suggest that the nauplius and adult stages feed on different resources. Comparing morphology interspecifically also revealed that the three sympatric species should have varying feeding habits in general, including filtering, scraping, and both combined, though *E. braueriana* has partial overlapping morphology with the other two species. This overlap may cause interspecific competition but may also allow *E. braueriana* expand its niche width when one or both resources are in short supply. Further studies are needed to know whether feeding morphology is consistent or adaptive to different environments.

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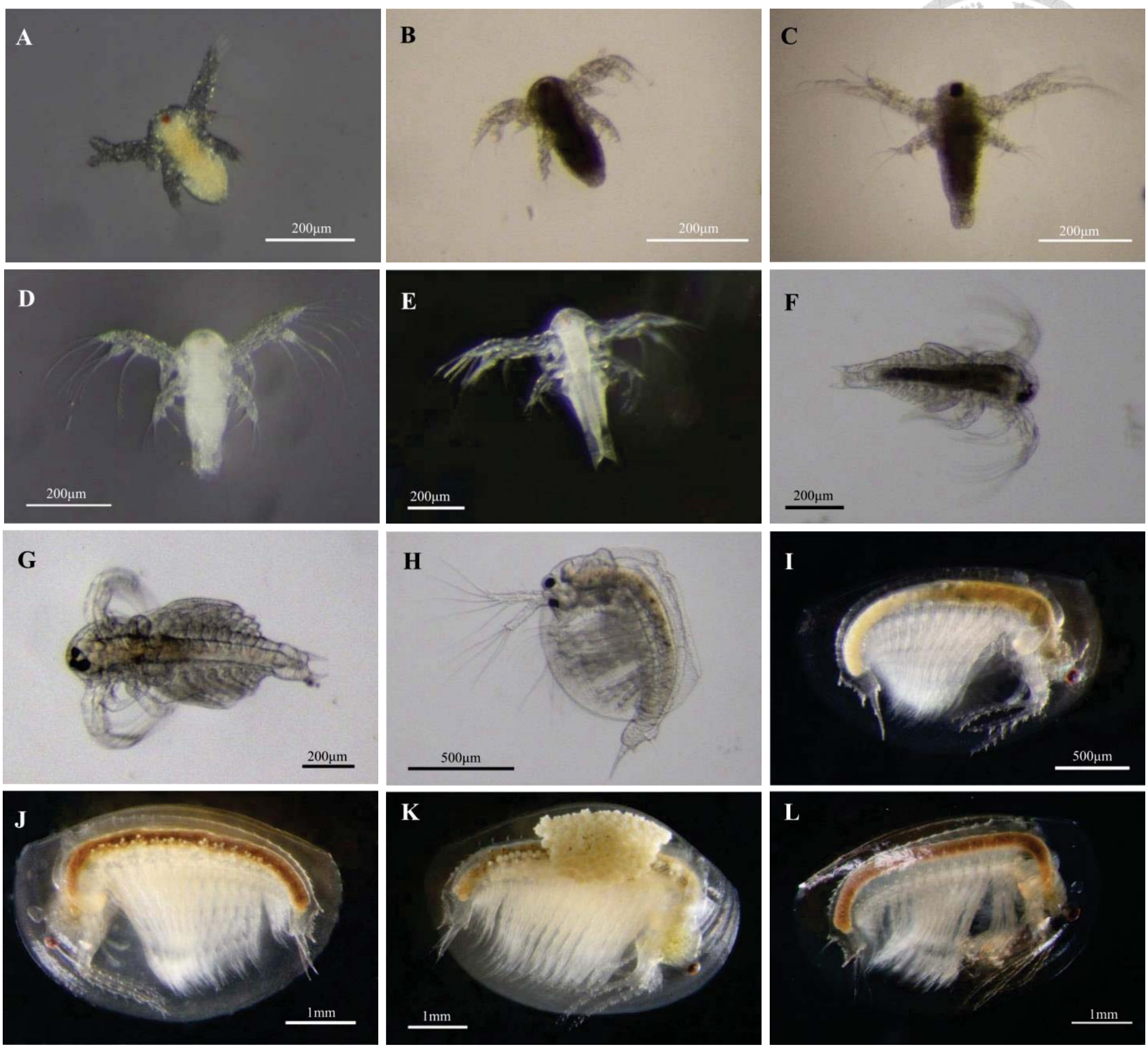

Fig. 1. Developmental stages of *Eulimnadia braueriana* observed under the light microscope. Juveniles and adults have similar morphology. A) Nauplius stage 1; B) nauplius stage 2; C) nauplius stage 3; D) nauplius stage 4; E) nauplius stage 5; F) nauplius stage 6; G) nauplius stage 7; H) early juvenile stage; I) late juvenile stage; J) early adult stage (hermaphrodite); K) fully grown hermaphrodite; L) fully grown male.

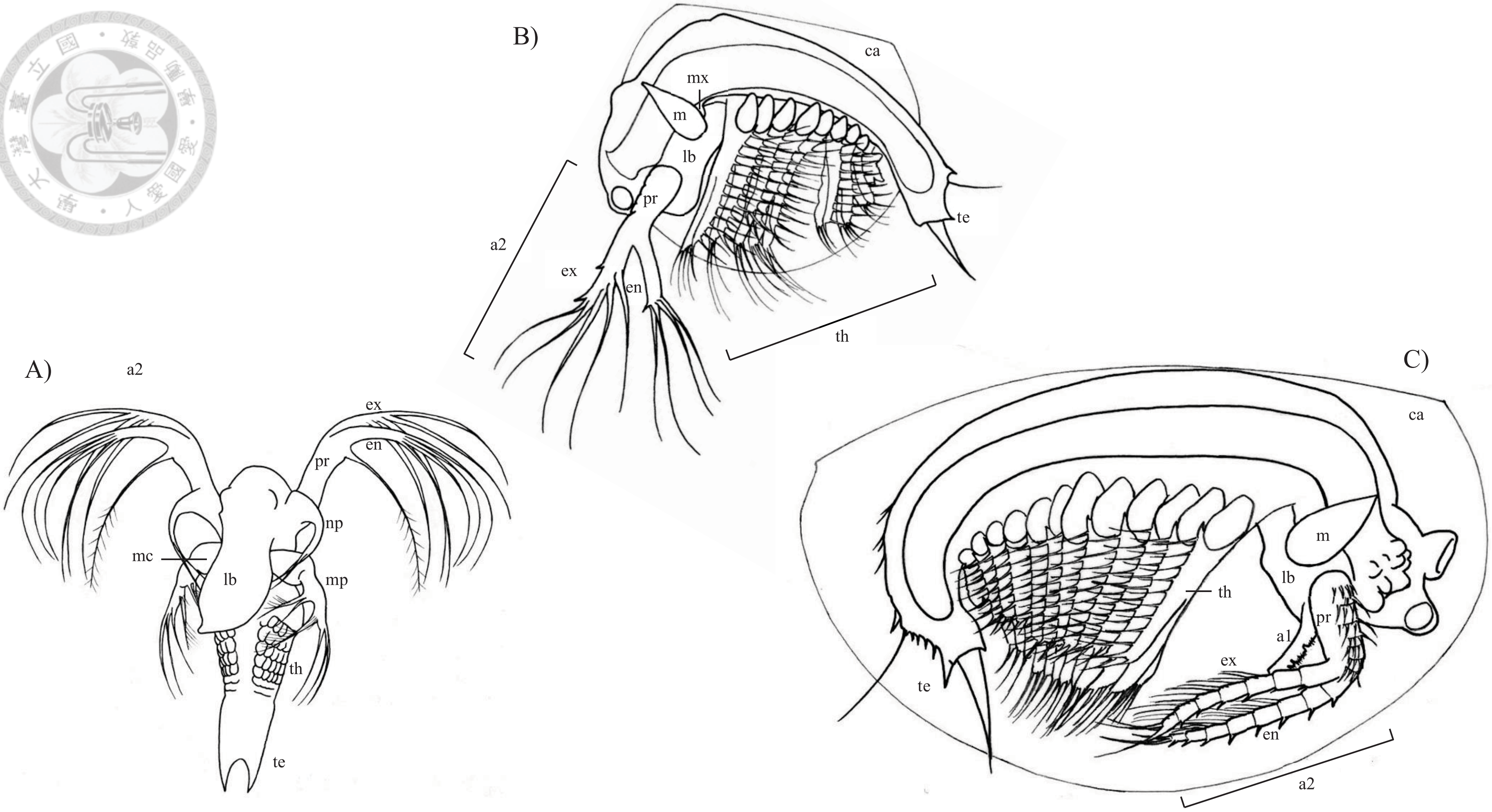

Fig. 2. *Eulimnadia braueriana* diagram. A) nauplius stage; B) early juvenile stage; C) late juvenile and early adult stage (structures for reproduction not implied). a1, first antenna; a2, second antenna; ca, carapace; en, endpod; ex, exopod; la, labrum; m, mandible; mc, mandible coxa; mp, mandible palp; np, naupliar process; pr, protopod; te, telson, th, thoracopod(s).

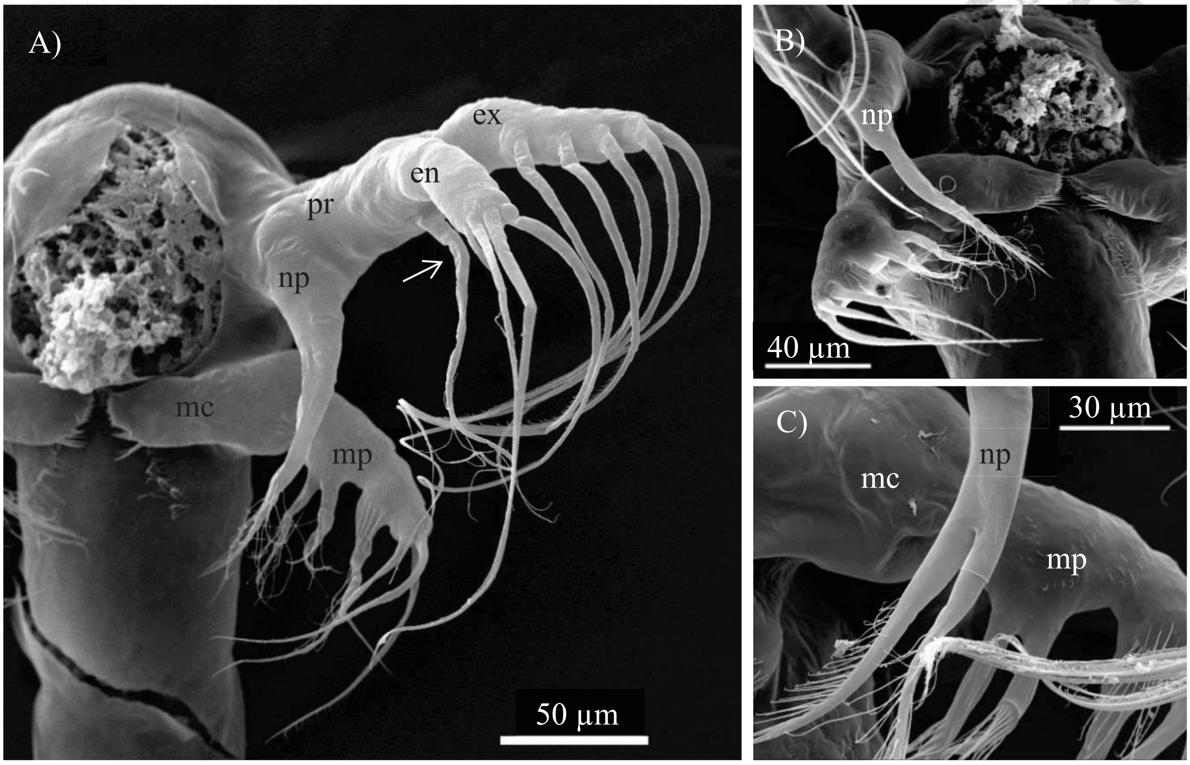

Fig. 3. Nauplius second antennae. The naupliar process and distal masticatory spine sweep food particles to the mouth during the anterior-posterior strokes that are also responsible for locomotion. A) Overall appearance of the second antenna (nauplius substage 3). The entangling of setae is an artifact. B) Naupliar process at nauplius substage 3. C) Bifid naupliar process after nauplius substage 4 (picture was taken from substage 6). en, endpod of second antenna; ex, exopod of second antenna; mc, mandible coxa; mp, mandible palp; np, naupliar process; pr, protopod of second antenna; arrow, distal masticatory spine

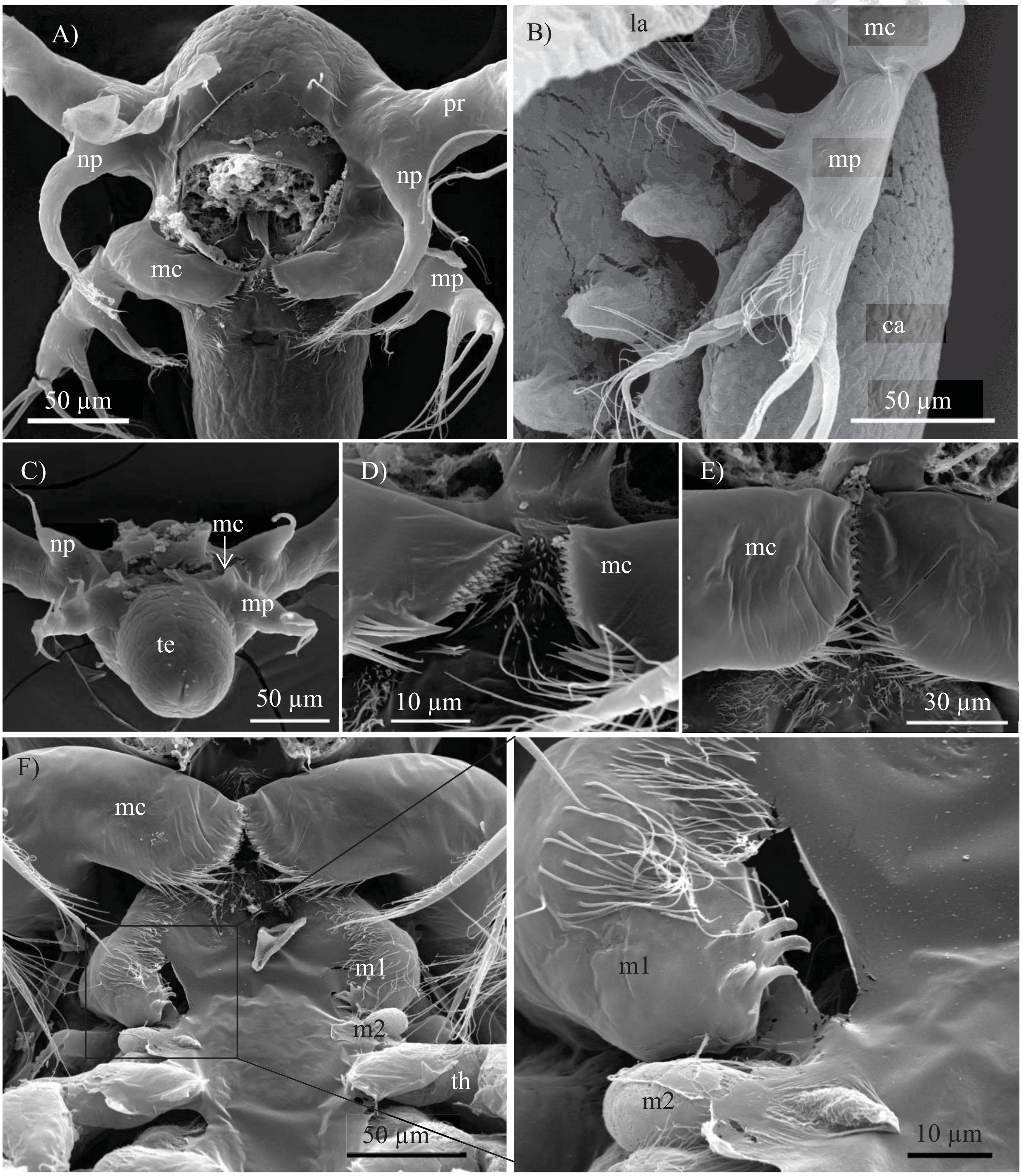

Fig. 4. Nauplius mandibles and maxillae. The mandibles meet at the midline in the fourth substage and maxillae just start to develop at the end of the nauplius stage. A) Relative locations of feeding structures (substage 2). B) Mandibular palp (substage 7). C) Mandible coxa at substage 1 (posterior view). D) Molar surface at substage 3. E)

Mandible coxa at the last nauplius substage (substage 7). F) Relative location of mandible and maxillae (substage 7). a2, second antenna; mc, mandible coxa; mp, mandible palp; ms, molar surface; mx1, first maxilla; mx2, second maxilla; np, naupliar process; pr, protopod; th, thoracopod.

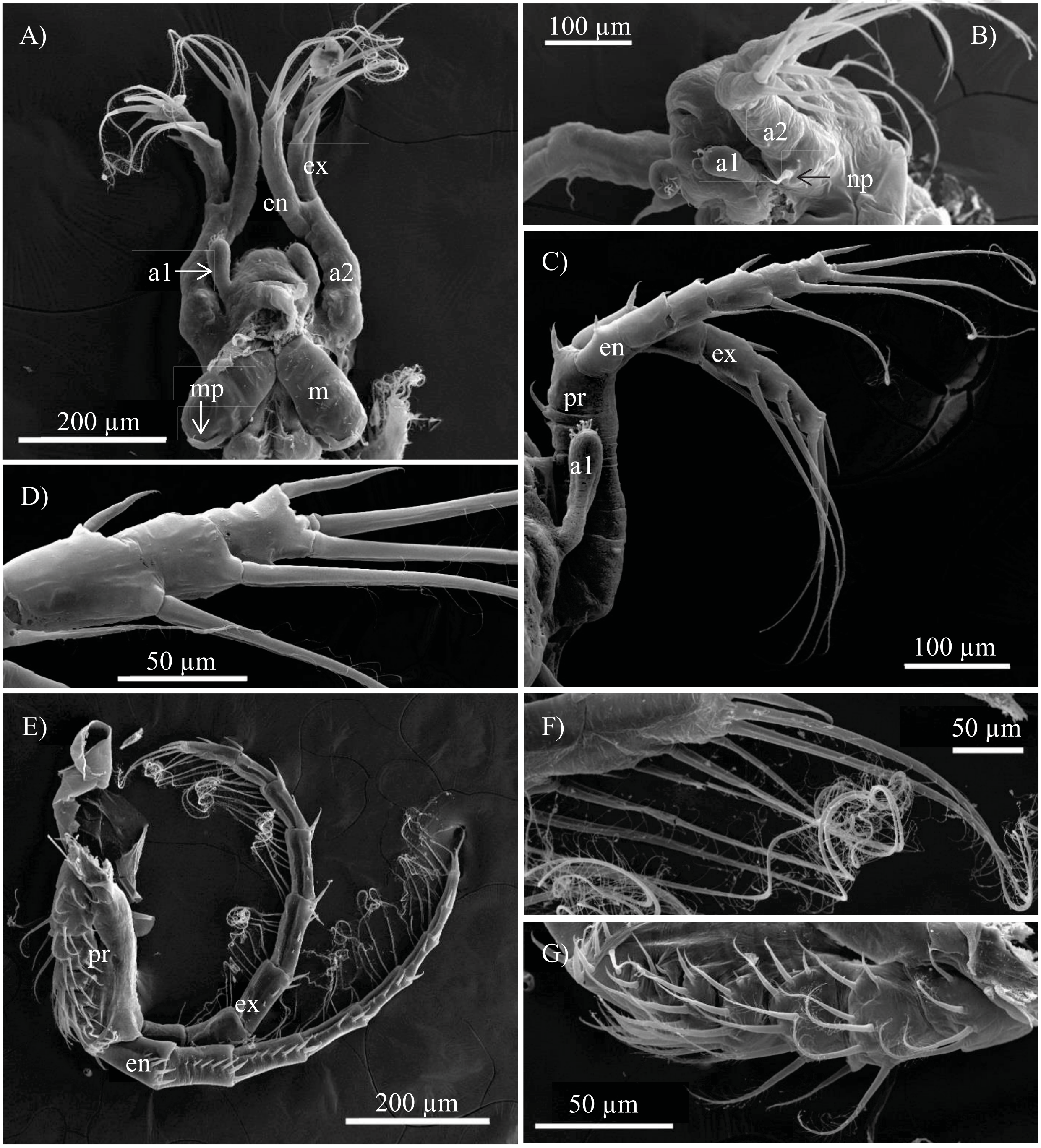

Fig. 5. Juvenile and adult second antennae. The masticatory spines degenerate in the juvenile stage. Adult antennae have more segments and setae. A) Early juvenile antenna (ventral view). B) Degenerating naupliar process. C) Late juvenile antenna without masticatory spines (ventral view). D) Endopod setae (late juvenile). E) Adult antenna

(medial view). F) Endopod setae (adult stage). G) Setae on the protopod. a1, first antenna; a2, second antenna; en, endpod; ex, exopod; m, mandible; np, naupliar process; pr, protopod.

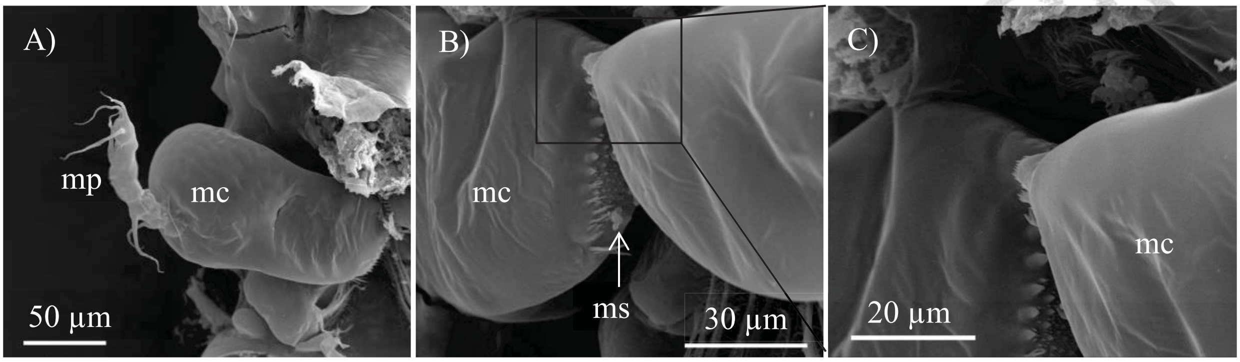

Fig. 6. Juvenile mandibles. The mandibular palp is degenerating and molar surface starts to differentiate. A) Degenerating mandibular palp (early juvenile, ventral view). B) Early juvenile molar surface with preliminary asymmetry. mc, mandible coxa; mp, mandible palp; ms, molar surface.

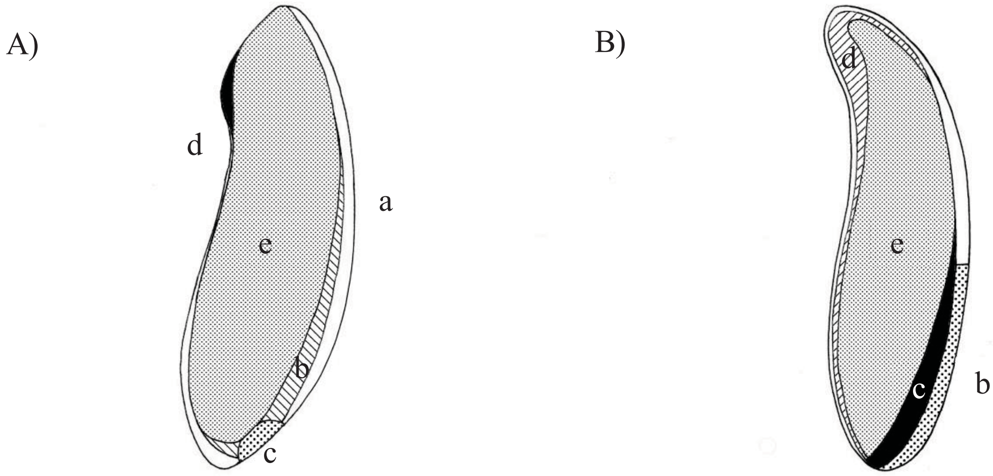

Fig 7. Diagram of adult right molar surface. Colors patterns represent similar morphology. A) Molar surface of *Eulimnadia braueriana*. B) Molar surface of *Branchinella kugenumaensis*. a, anterior bordering flagellated region; b, posterior bordering flagellated region; c, transitional zone; d, peripheral region; e, center region.

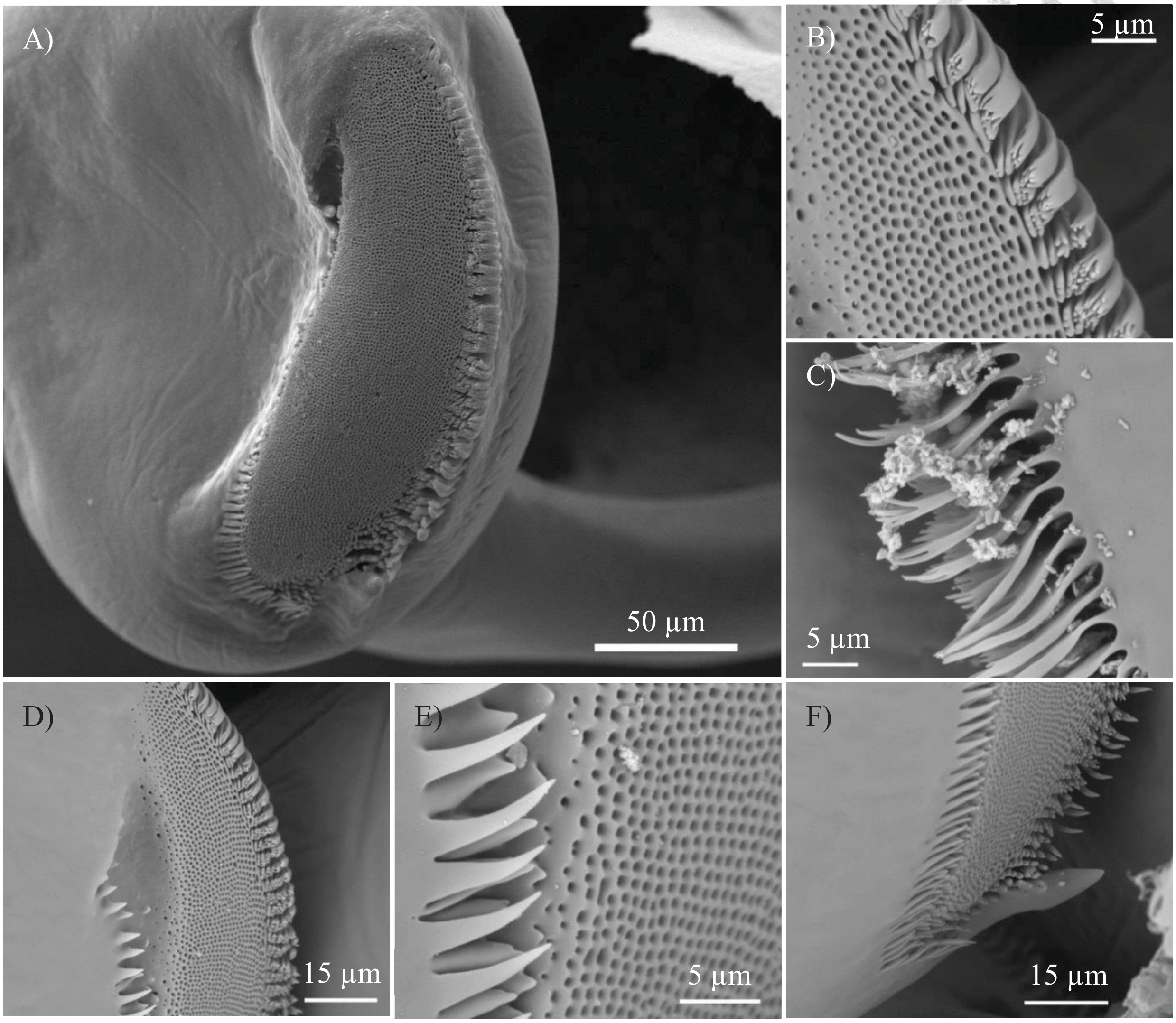

Fig 8. *Eulimnadia braueriana* adult molar surface. Molar surphase morphology is fully differentiated. A) Right molar surface. B) Anterior bordering flagellated region. C) Posterior bordering flagellated region. D) Anterior part of the molar surface. E) Peripheral region. F) Posterior tooth.

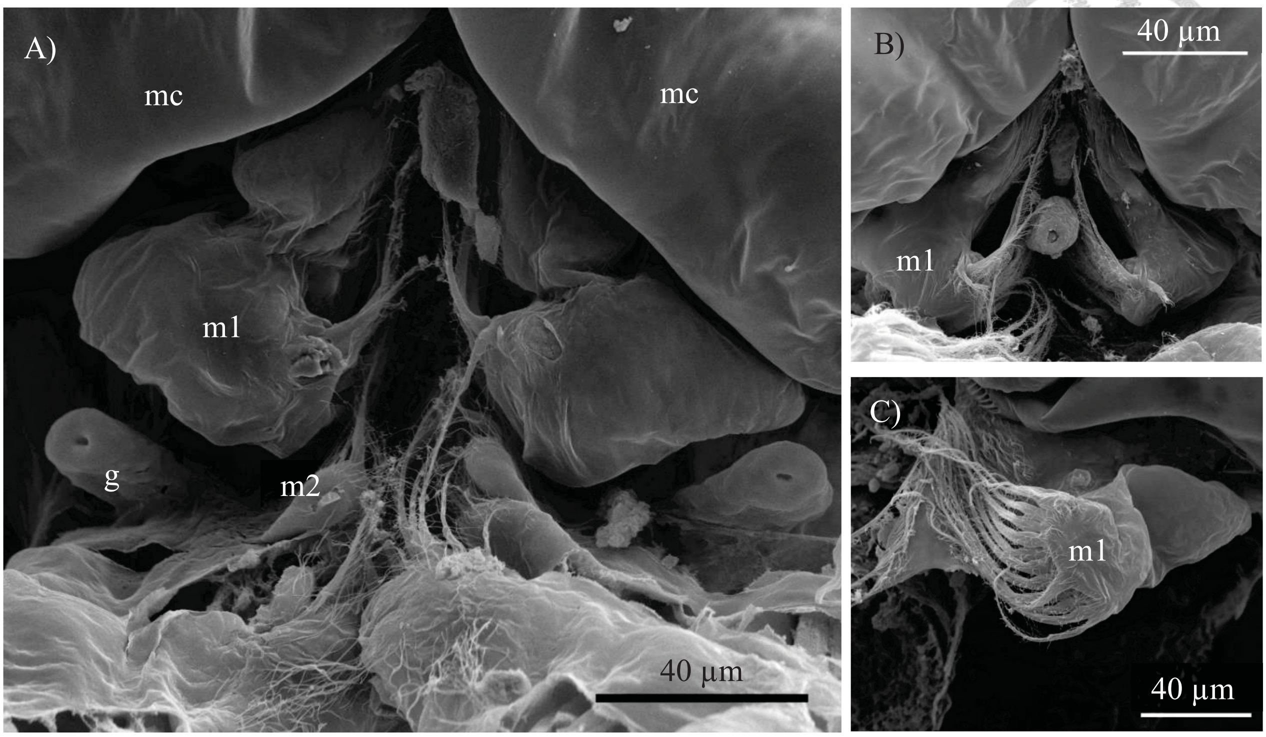

Fig. 9. *Eulimnadia braueriana* fully developed maxillae. Both first and second maxillae have dense setae that reach towards the mouth. A) Relative location of feeding structures. B) First maxillae with transported or blocked particles. C) Setae of the first maxillae. mc, mandible coxa; m1, first maxillae; m2, second maxillae; g, labral gland.

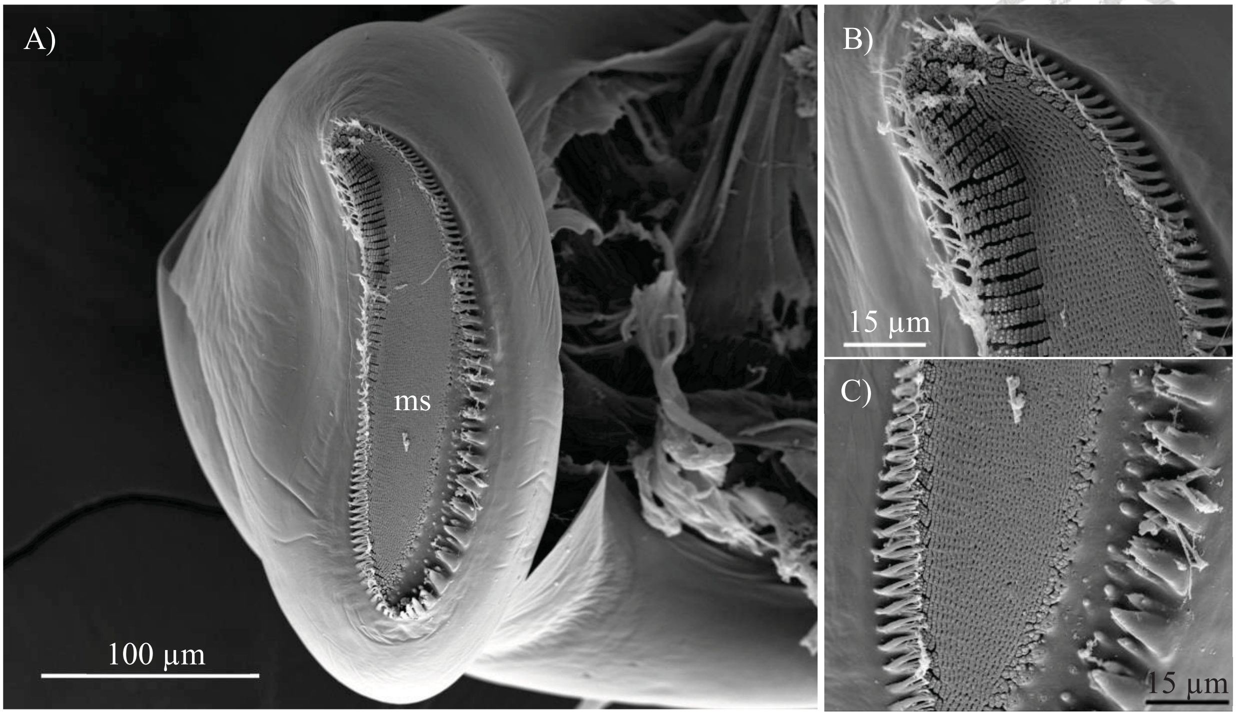

Fig. 10. *Branchinella kugenumaensis* molar surface. Spines and protrusions are distributed around the border while the central region is flat and perforated. A) Right molar surface (anterior is up). B) Anterior part of the molar surface. C) Posterior part of the molar surface. ms, molar surface.

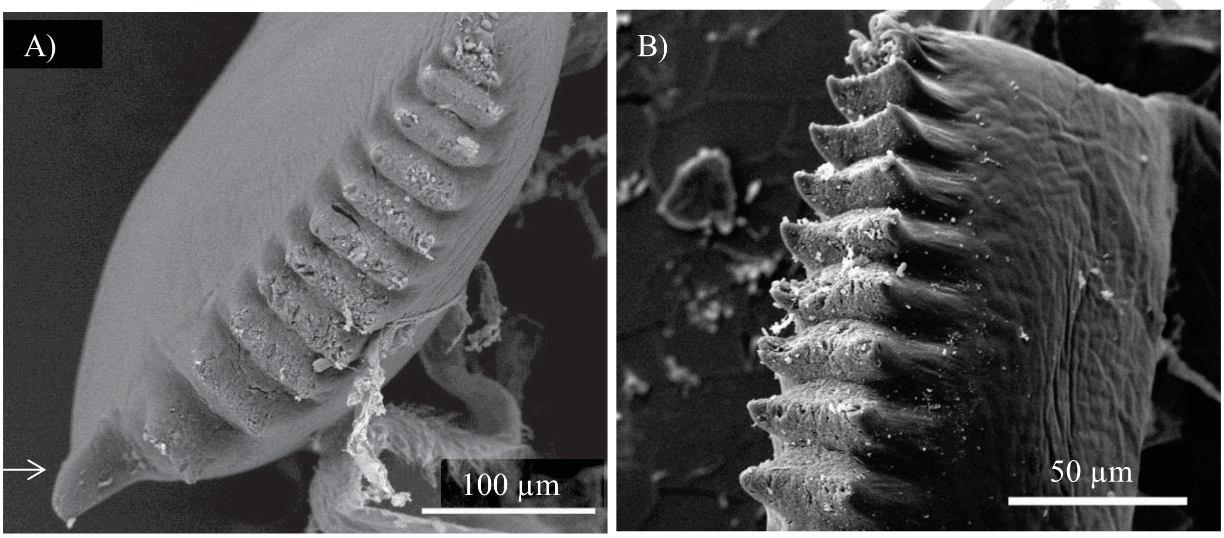

Fig. 11. *Lynceus biformis* molar surface. It consists of one posterior tooth and eleven dorso-ventral ridges. A) Right molar surface. B) Ridges of the molar surface. Arrow: posterior tooth.

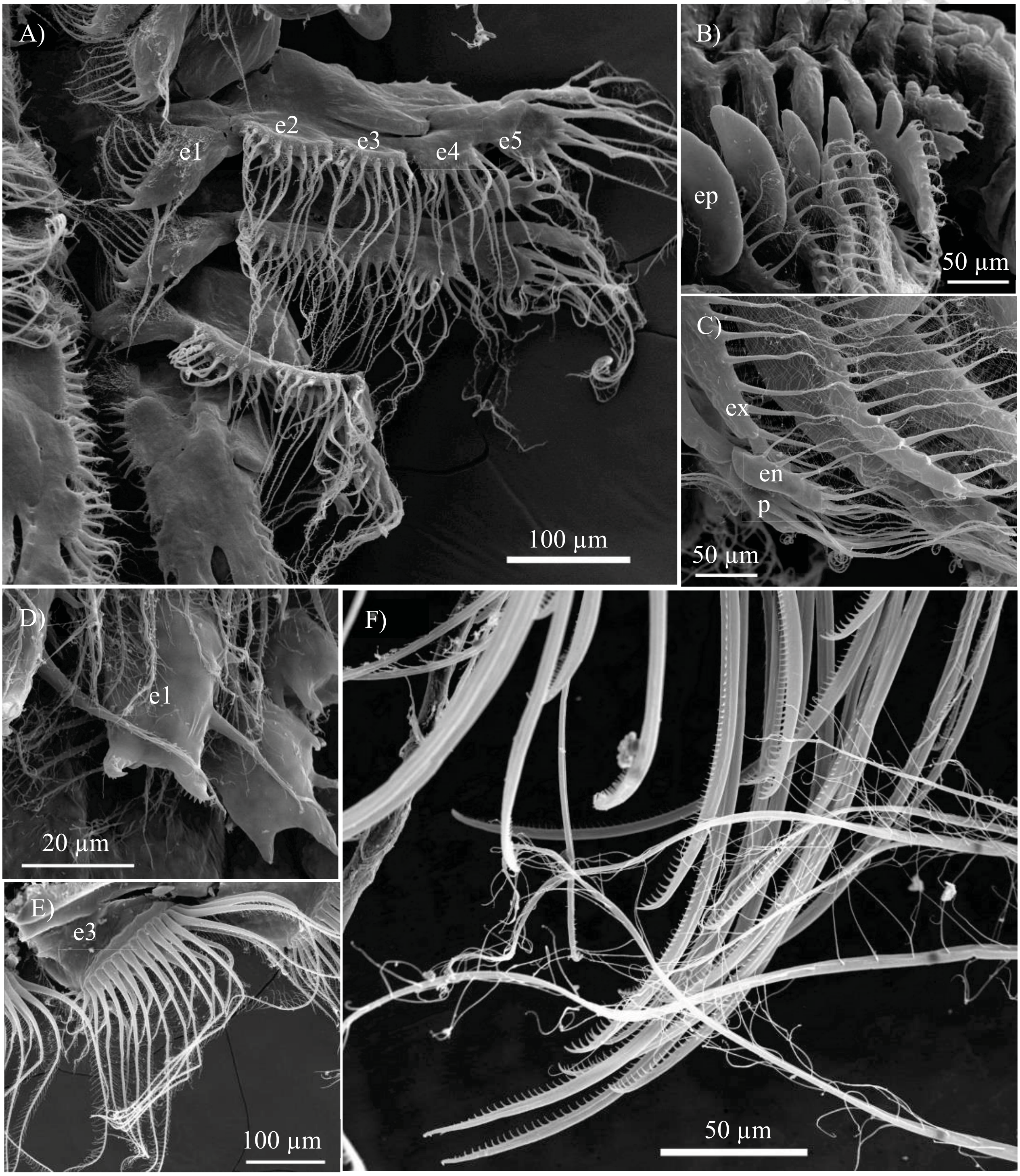

Fig. 12. *Eulimnadia braueriana* juvenile and adult thoracopods. Most endites have dense, plumose setae. Endite V of the first several thoracopod pairs also bears an elongated palp. A) Medial-anterior view. B) Lateral view: epipod and exopod. C) Lateral view of thoracopods: endopod and exopod. D) Anterior view of endite I (dorsal

is to the left) E) Setae of the second to fifth endite (anterior view). F) Scraping setae on the palp of endite V. e1-5, endite I-V; en, endopod, ep, epipod; ex, exopod; p, palp on endite V.

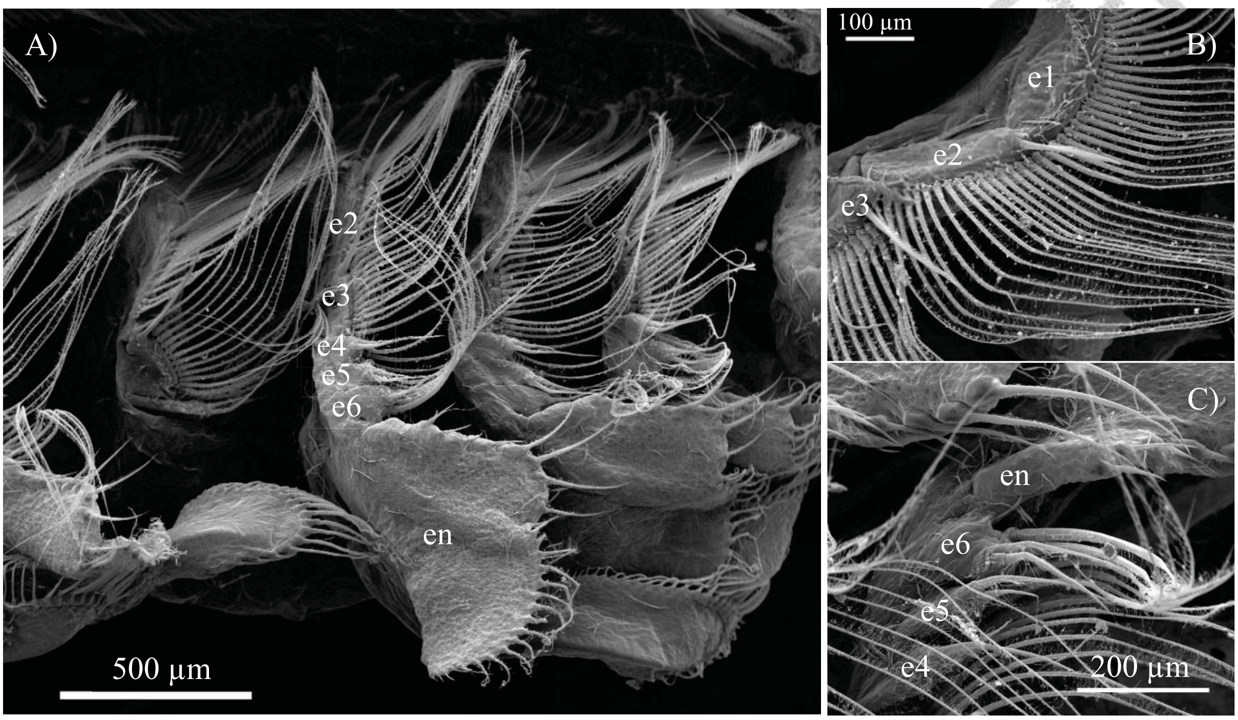

Fig. 13. *Branchinella kugenumaensis* thoracopods. Endites I-III bear long, dense setae. A) Medial-ventral view. B) Endites I-III. C) Setae of endites IV-VI and endopod. e1-6, endite I-VI; en, endopod.

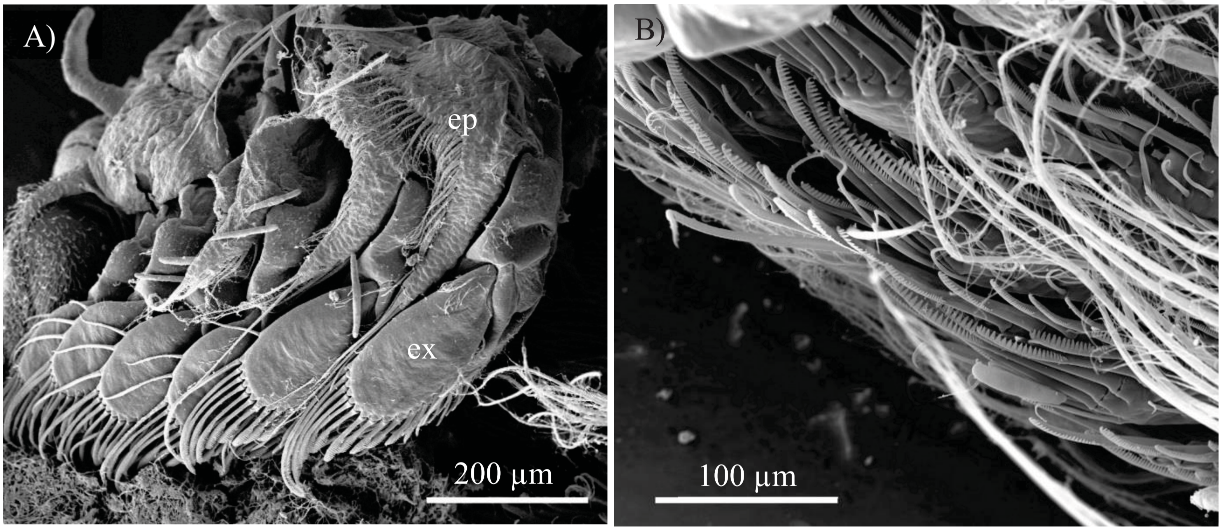

Fig. 14. *Lynceus biformis* thoracopods. Setae on the medial-distal end are dense and robust. C) Lateral view. D) Scraping setae (ventral view). ep, epipod; ex, exopod

Chapter 4: Grand conclusion

1. *Eulimnadia braueriana* phototactic tendency changed from positive to negative between the nauplius and juvenile stage. This indicates that *E. braueriana* will migrate from their hatching place to the surface at the nauplius stage, and then return to the bottom after the juvenile stage, showing a bottom-surface-bottom migration during development. Such migration could be beneficial in distributing evenly through the habitat, avoiding predators and feeding.
2. *Eulimnadia braueriana* naupliar feeding structures have collective function starting from naupliar substage four. Upon entering the juvenile stage, all naupliar feeding structures but the mandible coxa degenerate and are substituted by more posterior adult feeding structures. These include the scraping setae on the thoracopods. From morphology we imply that the nauplii are filter feeding while the adults could utilize both filter feeding and scraping mode.
3. Comparing adult feeding structures with sympatric large branchiopods showed that *Branchinella kugenumaensis* and *E. braueriana* share similar molar surface and thoracopod morphology except the scraping setae. *Lynceus biformis* and *E. braueriana* share similar thoracopod setation, with stronger scraping setae in *L. biformis*, but have different molar surface morphology. *B. kugenumaensis* are filter

feeders and *L. biformis* are scrapers while *E. braueriana* could be both.

4. Combining phototaxis and morphology results, the most significant ontogenic changes occur at the same developmental stage, matching positive phototaxis with filter feeding in the water column and negative phototaxis with scraping or resuspending detritus at the bottom. In the adult stage, negative phototaxis seperates *E. braueriana* from the surface dwelling *B. kugenumaensis*. *L. biformis* and *E. braueriana* might have similar vertical distribution but molar morphology suggests some resource partitioning. Even when space is limited near the end of an inundation, the three sympatric species still have some niche differentiation. *E. braueriana* could face more competition due to partial overlap in feeding function, but wider feeding variety could also be beneficial when resource is limited.
5. This study provided basic information for future ontogenic studies. Combining it with further research on locomotion and reproduction could construct a systematic understanding of *Eulimnadia braueriana* living strategies. On a species level, further studies on cross population comparison are needed to determine which strategies are consistent and which are adaptive.

Appendix 1

Protocol for branchiopod SEM specimen preparation

1. Freeze specimen for 30 to 120 seconds based on the size of the specimen.
2. 5% formalin in 50% ethanol overnight at 4°C.
3. 50% ethanol for 15 min.
4. 60% ethanol for 15 min.
5. 70% ethanol for 15 min. (Could be stopped at this step for preservation.)
6. 80% ethanol for 15 min.
7. 90% ethanol for 15 min.
8. 100% ethanol for 15 min.
9. Repeat step 8.
10. Acetone for 15 min.
11. Repeat step 10.
12. Critical point drying with 3 to 4 cycles of CO_2 exchange.

Appendix 2.

Eulimnadia braueriana feeding structure development, earliest occurrence (hours after hatching) and assumed feeding method.

		Second antenna	Molar surface	Mandibular palp	Maxillae	Thoracopods	Carapace	Earliest occurrence (hr)	Feeding mode
Nauplius	I	Naupliar process without setules	Bud like	Smooth setae without setules	NA	NA	NA	0	Filter feeding
	II	Naupliar process with setules	Uniform spines	Setae of the first segment differentiated and directed to mouth part	Small setae on flat surface			1	
	III		Uniform spines at the anterior part, posterior tooth present, several rows of setae at the posterior part					1	
	IV	Naupliar process bifid						1	
	V					Bud like	Anlagen	3	
	VI						Covering to the first thoracopod pair	NA	
	VII				Small setae on elaborated surface pointing medially		Covering to the third thoracopods pair	12	
Juvenile	early	Naupliar process degenerating	Spines differentiating, left and right molar surface asymmetric	Degenerating	Dense plumose setae on both first and second maxillae	7 pairs	Covering the entire body. Without growth lines.	20	Filter feeding and scraping
						8 pairs		28	
						9 pairs		36	
	late	Naupliar process absent		NA		10 pairs		64	
						11 pairs		84	
Adult		Naupliar process absent	Spines fully differentiated, left and right molar surface asymmetric	NA	Dense plumose setae on both first and second maxillae	12-18 pairs with comb-like structure on the first several pairs	Covering the entire body. With growth lines.	120	