Alternative antipredator responses of two coexisting *Daphnia* species to negative size selection by YOY perch

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Our study showed for the first time in nature that two coexisting *Daphnia* adopted alternative life history and behavioural strategies to cope with negative size-selection predation by gape-limited young-of-the-year (YOY) perch. We evaluated the phenotypic plasticity in life history and behavioural traits of two coexisting *Daphnia* species, *D. pulicaria* (2 mm) and *D. galeata mendotae* (1.4 mm), in response to seasonal changes in predation by YOY yellow perch (*Perca flavescens*) in a mesotrophic lake. We expected that the large-sized *D. pulicaria*, the most likely subjected to size-selective predation by YOY perch, will show stronger antipredator responses than the small-sized *D. galeata mendotae*. To test this hypothesis, we examined changes in life history and behavioural traits in juveniles and adults of both species during four YOY fish predation periods that were selected based on the presence of YOY perch in the pelagic zone and the relative abundance of *Daphnia* prey in their gut contents. Our study supports the scenario of negative size-selective predation by gape-limited YOY perch on both *Daphnia* species. The electivity index indicated that no daphnids with a body length >1.75 mm were predated by YOY yellow perch. Coexisting *Daphnia* exhibited phenotypic plasticity in their antipredator defenses based on their vulnerability to seasonal changes in size-selective predation by YOY perch. Juvenile *Daphnia* were the targeted prey and they responded by a decreased body length. Behavioural defenses were the dominant strategy used by both adult *Daphnia* populations to withstand high predation. A decreased size at maturity was not employed by *Daphnia*, except at the very end of the predation period. Behavioural defenses are short-term strategy adopted to avoid predation. Both antipredator defenses became unnecessary expenses and were no longer sustained after the predation period.

INTRODUCTION

The phenotypic plasticity of life history or behavioural defenses developed by *Daphnia* species in response to risk of predation by planktivorous fish has already been extensively investigated under laboratory conditions (Boersma et al., 1998; de Meester et al., 1999; Tollrian and Dodson, 1999; Lass and Spaak, 2003). Visually hunting fish tend to select energetically more rewarding conspicuous large-sized *Daphnia* over smaller ones (Brooks and Dodson, 1965; Riessen, 1999; Tollrian and Dodson, 1999). Therefore, changes in life history traits, especially decreasing size and age at maturity associated with lower investment in growth and higher reproductive effort (Stibor, 1992) can increase fitness of *Daphnia* populations under positive size-selection by planktivorous fish. Another way of avoiding fish predation by *Daphnia* is to develop behavioural defenses such as depth selection and diel vertical migration (DVM) (Lampert, 1993; de Meester et al., 1999). Inhabiting the colder and darker deep strata of the water column during daytime allows *Daphnia* to decrease the risk of predation by visually hunting planktivorous fish (Lampert, 1993).
Fish predation pressure varies in a predictable way during summer season with the spring recruitment of new-hatched fish [young-of-the-year (YOY)] and their ontogenic changes. Prey selection changes during their growth, prey organisms may develop multiple alternative defenses depending on their vulnerability (Tollrian and Dodson, 1999). The relative importance of these alternative antipredator defenses for *Daphnia* populations facing YOY fish predation in nature has been less studied (Sakwinska and Dawidowicz, 2005). Nonetheless, it is now recognized that both life history strategies and depth selection behaviour are mutually adjusted alternative ways for *Daphnia* to cope with fish predation (de Meester and Weider, 1999; Sakwinska and Dawidowicz, 2005). However, most of the evidence comes from experimental studies (de Meester et al., 1995; Winder et al., 2004; Hülsmann et al., 2004), and there are still few studies considering the relative importance of life history and behavioural antipredator defenses for *Daphnia* populations in lakes where factors other than predation, such as food quantity and quality, and temperature gradients can blur the expected inducible responses (Sakwinska and Dawidowicz, 2005). Furthermore, studies examining how these antipredator strategies are employed by coexisting *Daphnia* having different size and predator vulnerability have still been limited to enclosure experiments (Bernot et al., 2004).

The present study investigates in nature how both life history and behavioural defensive strategies are associated in two coexisting *Daphnia* species of different size facing seasonal changes in planktivory by YOY yellow perch (*Perca flavescens*). The coexisting species are the large-sized *D. pulicaria* (2 mm) and its smaller congener *D. galeata mendotae* (1.4 mm) inhabiting a mesotrophic lake. In early summer, YOY perch feed mainly on zooplankton before switching to benthic prey and this seasonal pattern is generally associated with a drastic decline in *Daphnia* densities (Mills and Forney, 1983; Persson, 1986; Roseman et al., 1996; Johnson and Kitchell, 1996). Therefore, a question exists if daphnids were temporarily vulnerable to negative size-selective predation by new-hatched YOY perch which are limited by their gape dimension to eating small-sized prey (Hülsmann et al., 2004)? Our main hypothesis is that the coexisting *Daphnia* species will show phenotypic plasticity in their antipredator defenses and adopt different life history and behavioural strategies to cope with changing YOY perch size-selective predation. Positive size selection by large YOY perch would induce a stronger response in terms of life history and behavioural defenses in the large *D. pulicaria* than in the small *D. galeata mendotae*. To test these hypotheses, we compared the antipredator life history and behavioural strategies developed during the summer season by juveniles and adults of the two *Daphnia* species in relation to their different size and vulnerability to YOY perch predation.

**METHOD**

**Study site and field sampling**

Lake Brone is a large (14 km²), dimictic lake of glacial origin located in the Eastern Townships region, southeast of Montréal, Québec, Canada (45°14’N, 72°30’W). Mean and maximum depths are 5.9 and 12.2 m, respectively. The lake is normally ice-free from early April through late November. The lake is mesotrophic with maximum concentrations of 19 µg L⁻¹ for total phosphorus and 8 µg L⁻¹ for chlorophyll a.

Sampling was carried out every 4–10 days from May through August 2002 at three sampling stations, situated 200 m apart at the deepest site (12 m) of the lake using GPS navigation. All measurements and water sampling were done during the day (12h00–17h00). Water temperature and oxygen profiles were measured with an YSI-54A temperature-oxygen meter at 1 m intervals. Vertical profiles of light intensity were also measured at 1 m intervals with a LiCor-1000 quantum meter, and water transparency was recorded with a Secchi disk. An integrated water sample was collected within the euphotic zone (above 1% surface light irradiance: 0–9 m) using a one-way foot valve. Chlorophyll a depth profiles were measured with a bbe Moldaenke FluoroProbe (Beutler et al., 2002). In addition, 250 mL water subsamples were collected and preserved in 1% acid Lugol solution for phytoplankton identification.

To analyse the crustacean zooplankton community and *Daphnia* traits, zooplankton samples were collected during daytime with a plankton net (28 cm diameter, 150 µm mesh size) by vertical hauls in the water column (0–12 m) at each sampling station. The mesh size used in this study was small enough to collect most crustacean species, especially juvenile and adult daphnids, while filtering small rotifers and algae. To examine *Daphnia* behavioural traits, additional zooplankton samples were collected at six depths (1, 3, 5, 7, 9 and 11 m) during day (12h00–17h00) and night (22h00–00h00) with a Schindler–Patalas sampler (12 L) fitted with a 150 µm mesh size net. A total volume of 24 L was filtered at every depth. Zooplankton samples were fixed in the field in 4% sugar buffered-formalin solution (Haney and Hall, 1973).

YOY yellow perch (*P. flavescens*) were collected in the pelagic zone every week from 23 May until 29 July. A large Bongo net (0.25 m² area, 6 m length and 1 mm
mesh size) was trawled in the center of the lake along 200 m transects at 1, 3 and 5 m depths 2 h after sunset (between 20h00 and 22h00 depending on sunset time). The collected fish were identified, counted, measured and their gape width was estimated based on their body length using Schael et al. (1991) regression. We counted the total number of YOY perch captured at each sampling date to evaluate seasonal changes in their density (ind. m⁻³) during the survey. When fish were caught, at least 30 YOY fish specimens were fixed in methanol-formaldehyde solution immediately after catching, at least 30 YOY fish specimens were fixed in methanol-formaldehyde solution immediately after fishing and kept for further analysis of their gut content.

**Plankton analyses**

Phytoplankton samples were analysed only at four dates corresponding to the fish predation periods (see YOY planktivory analysis below) using an inverted microscope (Utermöhl method) to evaluate the density (10⁶ cells L⁻¹) of major taxonomic groups and dominant species. A sub-sample (5 or 10 mL) of each zooplankton sample collected with the plankton net in the water column was analysed under a dissecting microscope using a Ward counting wheel to determine the crustacean community composition. We estimated the density (ind. L⁻¹) of main cladoceran and copepod taxa (including the two *Daphnia* species) based on the relative percentage of the total volume of the sample analyzed and the volume of lake water filtered. Morphometric traits of the two *Daphnia* populations (*D. pulicaria* and *D. galeata mendotae*) were examined on around 50 daphnids in each zooplankton sample collected with the plankton net. The animals were examined at ×100 magnification with a dissecting microscope (Leica MZ12) and measurements were taken with an image analyser (Image Pro Plus). Body length was defined as the length between the top of the compound eye and the base of the tail spine, body width was defined as the largest distance between a ventral and a dorsal horizontal line (Tollrian, 1994). Size at maturity (SAM) was evaluated according to Stibor and Lampert (1993). The clutch size was estimated by counting the number of eggs per gravid parthenogenetic female. We also recorded the presence of ephippium in gravid *Daphnia* in each sample. In zooplankton samples collected at discrete depths with the Schindler–Patalas sampler, we identified and counted juvenile and adult of the two *Daphnia* species to estimate their weighted mean depth (WMD) during day and night, and the amplitude of their DVM.

\[
WMD = \sum \frac{N_i d_i}{N_i}
\]

where \(N_i\) is the number of *Daphnia* at the depth \(i\), and \(d_i\) is the depth \(i\) when \(i = 1, 3, 5, 7, 9, 11\) m.

The amplitude of the DVM was estimated as the difference between WMDs of the day and night samples.

**YOY planktivory analysis**

The summer survey was divided in four YOY fish periods according to the presence/absence of YOY perch in the pelagic zone of the lake and the abundance of *Daphnia* prey in their gut contents: the before-predation (BP) period, the low-predation (LP) period, the high-predation (HP) period and the after-predation (AP) period. The BP period (27 April to 31 May) corresponds to the absence of fish larvae in the pelagic zone of the lake as no YOY fish were caught in the Bongo net until the end of May. The LP period (1 to 16 June) corresponds to the presence of YOY fish which were caught in high numbers (3–6 ind m⁻³) in the pelagic zone; however, they did not significantly feed on *Daphnia* but mainly on *Bosmina* sp. and small calanoid copepods. The HP period (17 June to 15 July) represents the period of lower abundance of YOY fish but of important predation pressure on both *Daphnia* species which were the dominant prey in the gut contents during most of the period. The period AP (15 July to 19 August) is the AP period when YOY fish were not caught anymore in the pelagic zone. We analyzed the entire length of the gut content of YOY perch collected on each sampling date during the LP and HP periods. We identified and counted all prey organisms including crustacean zooplankton species, detached eggs of copepods and other invertebrates. All *D. pulicaria* and *D. galeata mendotae* observed in gut contents of YOY perch from 16 June to 15 July were counted and measured. The percentage of different prey items was calculated based on numbers of each prey item in the gut contents relative to the total number of prey. We compared the size distribution of *Daphnia* collected in the lake and *Daphnia* found in the gut contents of YOY perch. Size classes ranged from 0.75 to 2.25 mm with an increment of 0.25 mm. YOY perch feeding selectivity relative of other *Daphnia* species was estimated using the electivity index *Ei* (Ivlev, 1961) based on the proportion of each *Daphnia* size class in the gut content \(r_i\) and in the lake \(p_i\):

\[
E_i = \frac{r_i - p_i}{r_i + p_i}
\]
Statistical analyses

Repeated-measured ANOVAs were used to test for differences in the mean body length of adult and juvenile of each *Daphnia* species. The mean SAM and the mean clutch size of each *Daphnia* species were also analysed with repeated-measured ANOVAs to test for differences along the summer survey. Data were normally distributed according to Kolmogorov–Smirnov tests (*P* > 0.05) (Lilliefors) (Sokal and Rolf, 1995). Differences among the four YOY fish periods in WMD during day and night for the juvenile and adult of each *Daphnia* species were analysed using Kruskal–Wallis tests (Zar, 1999). The amplitude of the DVM pattern was estimated during the HP period based on differences in WMD during day and night. We tested if DVM amplitude was significant during the HP period using paired *t*-tests for differences between day and night WMD in juveniles and adults of both species. All computations were run with Systat 8 (Wilkinson, 1999).

RESULTS

Limnological conditions and plankton community composition

Following ice-melt, spring water mixing in May maintained an isothermal water column, which gradually warmed from 10°C in April to 15°C in early June (Fig. 1A). Thermal stratification developed during June and the lake was stratified from mid-June until the end of the survey. Water temperature in the epilimnion increased from 10°C in spring to 24°C in late summer, while hypolimnetic waters remained at 15–17°C. Lake Brome was well oxygenated (11 mg L⁻¹) over the entire water column in early May and in the epilimnion (>9 mg L⁻¹) during summer (Fig. 1B). Oxygen concentration in the hypolimnion began to decrease in June below 9 m depth and remained anoxic throughout the end of summer (<1 mg L⁻¹) after 10 July. Secchi disk depth was consistently around 3 m, and the euphotic depth remained around 9 m throughout summer.

In general, total chlorophyll *a* concentrations were always low in surface waters (0–1 m: < 5 μg L⁻¹), and reached a maximum of 8 μg L⁻¹ in the epilimnion on 16 June (Fig. 1C). Thereafter, chlorophyll *a* concentrations decreased throughout the summer until a minimum of 2–3 μg L⁻¹ was reached in August. Phytoplankton density was the highest in early summer around 2.63 x 10⁶ cell L⁻¹ during the BP period and 1.46 x 10⁶ cell L⁻¹ during the LP period. Chrysophytes (*Dinobryon divergens* and *Mallomonas pumilio canaensis*) and...
large diatoms (*Asterionella formosa* and *Melosira ambigua*) composed almost 100% of the phytoplankton community in spring (Fig. 2). Later in summer, densities of phytoplankton decreased to around $0.39 \times 10^6$ cell L$^{-1}$ and $0.55 \times 10^6$ cell L$^{-1}$ during the HP and AP periods, respectively (Fig. 2). The phytoplankton community was dominated by filamentous cyanobacteria (83–97% of total cell density) such as *Anabaena flos-aquae*, *Anabaena spiroides* and *Aphanizomenon gracile*, and the small diatom *Cyclotella glomerata* represented only 16 and 3% of total phytoplankton abundance, respectively (Fig. 2).

In spring, during the BP period, zooplankton densities reached around 700 and 400 ind L$^{-1}$ and the crustacean community was dominated by calanoid and cyclopoid copepods (Fig. 2). Among the cladocerans, three dominant taxa were present at low density: *Bosmina* sp., *D. pulicaria* and *D. galeata mendotae*. At the beginning of the LP period, zooplankton abundance was still high (around 700–800 ind L$^{-1}$), and the community dominated by copepods. However, *D. pulicaria* density increased up to 170 ind L$^{-1}$, and the density of *D. galeata mendotae* reached 85 ind L$^{-1}$ on 1st June. The density of total zooplankton and both *Daphnia* species decreased drastically at the end of the LP period. During the HP period, zooplankton density varied between 300 and 500 ind L$^{-1}$, and *D. pulicaria* accounted for a higher proportion of the community. At the end of the HP period, *D. pulicaria* abundance decreased to a minimum of 20 ind L$^{-1}$, and *D. galeata mendotae* dropped to less than 5 ind L$^{-1}$, suggesting that both *Daphnia* species were affected by YOY perch predation (Fig. 2). In the AP period, the density of *D. pulicaria* stayed low at 10 ind L$^{-1}$. In contrast, the density of *D. galeata mendotae* greatly increased and reached a maximum of 105 ind L$^{-1}$ on 19th August. At one date during the AP period, *Bosmina* was the dominant cladoceran (Fig. 2).
Seasonal changes in YOY fish planktivory

YOY perch (P. flavescens) was the dominant species captured during the survey; YOY yellow perch represented 99% of the samples. As stated above, the BP period was defined by absence of perch larvae in the pelagic zone. During the LP and HP periods, their density varied between 0.25 and 6 ind m$^{-3}$ (Fig. 3A). New-hatched YOY perch had a mean length of 8 mm, and by the end of the HP period, they reached an average 27 mm in total length (Fig. 3A). The highest densities (3–6 ind m$^{-3}$) were observed when YOY fish were smaller than 15 mm. Lower densities (<3 ind m$^{-3}$) were observed when YOY fish reached lengths greater than 15 mm. The low density on 24 June could be explained by the period of full moon when fish avoid the pelagic zone because moonlight makes them more vulnerable to their predators (Gliwicz, 1986; Gaudreau and Boisclair, 2000). During the AP period after mid-July, we did not collect any more YOY yellow perch in the pelagic zone; this was also confirmed by hydroacoustic data of the water column (Słusarczyk et al., in preparation). Either the sampling was inefficient for catching larger YOY perch which, by having greater swimming capacity, could escape the large Bongo net, or larger YOY perch moved to the littoral zone of the lake to escape piscivorous fish (Okun et al., 2005).

Gut content analysis showed important seasonal changes in the diet of YOY fish during their summer growth in the pelagic zone (Fig. 3B). For the first 2 weeks of June, the diet of new-hatched YOY perch (<15 mm) was mainly composed of calanoid copepods and eggs, and of the small cladoceran Bosmina; their gape width was too small to capture daphnids (Fig. 3C). When YOY perch reached a length of 15 mm with a gape width larger than 2 mm in mid-June, they began to feed on Daphnia, selecting a small proportion of both the small-sized D. galeata mendotae and the large-sized D. pulicaria. The large-sized D. pulicaria became the predominant item in the diet of YOY perch later at the end of June and the beginning of July when YOY perch measured between 15 and 27 mm. On 15 July, both Daphnia species were rarely found in the gut content of larger YOY perch which fed again on Bosmina and copepods.

The selectivity index indicated that YOY fish are limited by their small gape to the smaller zooplankton because they started by feeding on the very small juveniles (0.75 mm) of both Daphnia species on 16 June when they were smaller than 15 mm (Fig. 4). Moreover, they only fed on Daphnia prey with a maximum size of 1.5 mm in July when they reached larger length (15–27 mm), although larger Daphnia (1.5–2.5 mm) were present in the lake. Ivlev’s selectivity index values indicated that small juveniles (0.75–1 mm) of both Daphnia species were the only Daphnia prey selected by very small YOY perch larvae (<15 mm) on 16 June, while Daphnia of medium size (1–1.5 mm) were selected later in June and in July when YOY fish grew to greater length (≥15 mm) (Table I). Daphnia prey of size greater than 1.75 mm were never found in the gut contents of YOY perch caught in the pelagic zone during the survey (Table I).

Life history traits

The mean body length of D. pulicaria was always larger than that of D. galeata mendotae in both adults and juveniles (Fig. 5). For both Daphnia species, repeated-measured ANOVAs indicated significant changes in body length of adults and juveniles among dates during the summer survey (Table II). For adults of D. pulicaria, there was a significant tendency for a smaller mean body length at the very end of the HP period on 15 July (Fig. 5). The mean body length of juveniles of D. pulicaria was smaller during the entire HP period (Fig. 5). The mean body length of D. galeata mendotae was least at the end of the HP period in both adults and juveniles. The decrease in body length for D. galeata mendotae at the end of the HP period was less pronounced than for D. pulicaria (Fig. 5).

The SAM changed significantly during the summer survey (Table II). SAM was the smallest at the very end of the HP period on 15 July for both species (Fig. 6). Daphnia pulicaria showed the strongest reduction in SAM (1.75–1.35 mm), whereas the response of D. galeata mendotae was weaker (1.2–1 mm).

Clutch size of D. pulicaria and D. galeata mendotae varied significantly among dates during the survey (Table II). It was the highest during the BP period when YOY perch were not yet present in the pelagic zone of the lake (Fig. 6). During the other periods (LP, HP, AP), D. pulicaria clutch size decreased to values around or lower than 2 eggs per female. Daphnia galeata mendotae clutch size declined as low as 1.5 eggs per gravid female at the end of the LP period, then increased during the HP period to 2.5 eggs, and reached values around 4 eggs during the AP period (Fig. 6). Only, the large-sized D. pulicaria produced ephippia during the LP and HP periods. The first ephippium was recorded on 1 June and resting egg production was an active phenomenon until 15 July.

Behavioural traits

Day and night WMD and DVM amplitude of D. pulicaria and D. galeata mendotae were determined for juveniles and adults separately (Fig. 7). Daytime WMD
of adults of both species changed significantly between the YOY fish periods (Table III), being clearly deeper during the HP period (Fig. 7). In contrast, nighttime WMD of adults of both species did not change significantly between the periods. The WMD of the juveniles of *D. pulicaria* was deeper during the HP period both
during day and night, whereas WMD of juvenile *D. galeata mendotae* did not change significantly during the HP period (Table III).

In spring (BP period) and at the beginning of the LP period, the WMD of juveniles and adults of both species were located at the mean depth of the lake.

**Fig. 4.** Size distributions of *D. pulicaria* and *D. galeata mendotae* found in YOY perch gut contents (white) and in zooplankton samples collected in the lake (black) from 1 June to 15 July. Size classes were established with an increment of 0.25 mm on the total range of size of *Daphnia* observed in our study.
Calculations based on the size distributions of each Daphnia species in the lake and in the YOY gut contents during the LP and HP periods.

Table I: Ivlev’s selectivity index of YOY perch for D. pulicaria and D. galeata mendotae of different size classes (mm) (−1 highly avoided, 1 preferred)

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<th>16 June</th>
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<th>28 June</th>
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DISCUSSION

Our field study shows important seasonal changes in size-selective predation of YOY perch associated with phenotypic plasticity in life history and behavioural traits of the two coexisting Daphnia species. Even though the impact of small YOY perch cannot be dissociated from the potential impact of larger planktivorous fish known to occur during summer in Lake Brome (Mason et al., 2004), YOY perch was the main visually hunting predator of Daphnia captured in the pelagic zone during our survey (Pierce et al., 1994). Generally, young fish of all species combined numerically dominates over larger planktivorous fish by several orders of magnitude after their hatching at spring (Wagner et al., 2004) and they are among the most important vertebrate zooplanktivores in lakes (Hülsmann et al., 2004).

Our study supports the alternative hypothesis of negative size-selective predation by small YOY perch, as observed by Hülsmann et al. (Hülsmann et al., 2004). Gape-limited feeding and negative size selection are predatory strategies already observed for YOY of yellow perch and Eurasian perch in previous field studies (Hansen and Wahl, 1981; Mayer and Wahl, 1997; Mehner et al., 1998; Graeb et al., 2004) and laboratory experiments (Hülsmann et al., 2004). An interesting result is that small YOY perch (<15 mm), restricted by their gape width, cannot prey on large body length and width of Daphnia species. Medium-size YOY perch (15–20 mm) and larger YOY perch (20–25 mm) showed negative size-selection on Daphnia species, selecting prey of size less than 1.5 mm, even though larger D. pulicaria prey were available in the lake. Such a threshold in the maximum size of Daphnia prey ingested by gape-limited YOY perch could also be attributed to a population-density threshold operating at both the individual and population levels (Gliwicz, 2001). Individuals selected by planktivorous fish tend to be more conspicuous not only in terms of their body size but also in terms of their abundance in the field (Gliwicz, 2001). In our study, a population-density threshold could explain foraging switch of YOY perch from D. galeata mendotae to D. pulicaria in late June and from D. pulicaria to Bosmina sp. in late July. On 15 July, Bosmina was the selected prey of large YOY fish (27 mm) probably because its population was the most abundant even though its body size was two times smaller than the daphnids which were in low numbers. The seasonal changes in size-selective feeding of YOY perch influence the succession pattern of coexisting Daphnia species in Lake Brome. Because larger YOY perch shifted their diet towards the slightly larger D. pulicaria during the HP period, D. galeata mendotae was released from predation pressure and could reach again high abundance after the fish predation period. In contrast, the heavily predated D. pulicaria produced diapausing eggs as the ultimate response to survive during the high predation period, and did not recover after the high predation period (Shusarczyk et al., 2005).

Common antipredator responses to positive size selection may be maladaptive responses to gape-limited predators which feed on small or medium-size prey.
(Hülsmann et al., 2004). In Lake Brome, the strong decrease in the body length of adults for both Daphnia species at the end of the HP period could have been caused by selective elimination of large bodied Daphnia by visually feeding fish or by the simultaneous maturation of smaller juveniles into small body size adult. However, the absence of large D. pulicaria prey in gut contents of YOY perch (no Daphnia prey > 2 mm) and their selective feeding on smaller specimens (with 1 mm Daphnia most eagerly eaten by the YOY perch) indicate negative size-selective predation of perch fingerlings. Clearly, the body size reduction of juveniles at the end of the HP period can be seen as disadvantageous when facing gape-limited YOY perch that selectivity fed on them, but may be a consequence of selective predation on the larger juveniles by growing YOY perch. Interactive effects of fish predation and algal food limitation may also explain seasonal changes in Daphnia body length (Ghadouani et al., 2003, 2006; Hülsmann et al., 2004). However, the food limitation interference is likely of low influence in Lake Brome because body size of both juveniles and adults of each species increased again as soon as YOY perch predation pressure is released while cyanobacteria were still dominant. Ślusarczyk et al. (Ślusarczyk, Pinel-Alloul and Gélinas, Warsaw University, Université de Montréal, 2004).

Fig. 5. Variation in the mean body length (mm) of adults and juveniles of D. pulicaria (top panel) and D. galeata mendotae (bottom panel) during the YOY fish periods (BP, LP, HP, AP) in summer 2002. Error bars on mean values correspond to the standard deviation.
Daphnia \textit{et al.} (2005) showed that removing fish-chemical signal \textit{SAM} increased markedly in the AP period. Mikulski reduction of \textit{SAM} observed for both species may be older planktivorous fish. Again, the short-term the season under positive size-selective predation by

phenomenon of the \textit{SAM} increase when YOY fish changes and mature at a larger size. In our study, the phenomenon of the \textit{SAM} increase when YOY fish disappeared from the pelagic zone after 15 July could be explained by this mechanism.

Both \textit{Daphnia} species had smaller clutch sizes during the LP and HP periods. Reducing clutch size may be beneficial for \textit{Daphnia} fitness by reducing prey visibility (Pearre, 2003). The reduction in clutch size was also associated with a shift in algal food resource from edible (Chrysophytes and diatoms) to inedible (cyanobacteria) algal food during the HP period. Another alternative explanation could be that \textit{Daphnia} decreased the energy allocated to fecundity to allow the production of other inducible defenses such as morphological defenses as a way to increase their long-term fitness, as reported by Boersma \textit{et al.} (1998) for clones of \textit{D. magna} and for the two coexisting \textit{Daphnia} species in Lake Brome (Gélinas \textit{et al.}, 2007). Seasonal variation in clutch size of the small \textit{D. galeata mendota} also supports the hypothesis of negative size-selective predation by the gape-limited YOY perch. As predation pressure on the small species declined during the HP period, its clutch size tended to increase and reached its highest value at the beginning of the AP period. As already mentioned, the large-sized \textit{D. pulicaria} adopted another life history response by producing ephippia while maintaining small clutch size in parthenogenetic individuals. In enclosure experiments with gape-limited small larval walleye (8-mm standard length), coexisting \textit{D. pulicaria} and \textit{D. galeata mendota} exhibited similar shifts in life history traits (Bernot \textit{et al.}, 2004), as observed in Lake Brome. It appears that the tendency to produce ephippia in spring is a characteristic associated with the large species most likely to be eliminated later in summer, as reported by Threlkeld (1979).

Deep-water habitat selection and DVM are well-known behavioural strategies used by \textit{Daphnia} to escape positive size-selective predation by visually hunting fish (Mills and Forney, 1983; Lampert, 1993; Ringelberg \textit{et al.}, 1997; de Meester \textit{et al.}, 1999). Our study indicates that a behavioural response is strongly induced in \textit{Daphnia} populations by the small YOY perch also. Intense predation by YOY yellow perch >15 mm during the HP period activated DVM behaviour and forced the juveniles and adults of both \textit{Daphnia} species to seek refuge in daytime in deeper and darker waters. Similar behavioural strategy was developed by \textit{D. hyalina x galeata} facing predation by YOY of Eurasian perch of size >15 mm (Ringelberg \textit{et al.}, 1997; Van Gool and Ringelberg, 2002). One interesting point is that the two coexisting \textit{Daphnia} species displayed similar habitat selection strategies while facing YOY predation: they both selected deeper habitat with lower light level. This result is contrary to what is usually observed, the large-sized \textit{D. pulicaria} tend to select deeper habitat than the

<table>
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<th>F</th>
<th>P</th>
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<td>0.033</td>
<td>6.498</td>
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unpublished data) measured the lipid content and the weight–length relationship parameters (intercept and slope) of \textit{D. pulicaria} and none of these indices suggested a potential food limitation.

The decrease in \textit{SAM} is another life history response to counteract positive size-selective predation by fish (Boersma \textit{et al.}, 1998; Riessen, 1999). However, the adaptive significance of a decreased \textit{SAM} in \textit{Daphnia} populations facing gape-limited predation by YOY fish has recently been questioned under experimental conditions (Hülsmann \textit{et al.}, 2004). Our study indicates that \textit{SAM} was relatively constant except at the very end of the HP period where a significant short-term reduction was observed in both \textit{Daphnia}. A lack of variation in \textit{SAM} life history trait in response to gape-limited predation has already been reported in enclosure experiments and laboratory studies for \textit{D. galeata} (Hülsmann and Mehner, 1997), \textit{D. magna} (Mikulski, 2000) and \textit{D. pulicaria} (Bernot \textit{et al.}, 2004). Hülsmann \textit{et al.} (2004) suggested that under field conditions, a lack of response in \textit{SAM} under negative size-selective predation early in the summer is advantageous for \textit{Daphnia} survival later in the season under positive size-selective predation by older planktivorous fish. Again, the short-term reduction of \textit{SAM} observed for both species may be due to the maturation of small juveniles. Interestingly, \textit{SAM} increased markedly in the AP period. Mikulski \textit{et al.} (2005) showed that removing fish-chemical signal can induce \textit{Daphnia} to withdraw from life history changes and mature at a larger size. In our study, the phenomenon of the \textit{SAM} increase when YOY fish disappeared from the pelagic zone after 15 July could be explained by this mechanism.

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Table II: Repeated-measured ANOVA testing for the differences among dates during the summer period in the body length of \textit{D. pulicaria} and \textit{D. galeata mendota}, respectively, for the juveniles and adults, and the \textit{SAM} and clutch size of adults.
small-sized *D. galeata mendotae* as observed in a large indoor enclosure experiment (de Meester *et al.*, 1995) and in field studies (Threlkeld, 1979; Leibold and Tessier, 1991, 1998; Tessier and Leibold, 1997). Possible explanations could include a large bin width allowing movement within the bins. Thus, one species could have a mean depth only slightly below the other, and it might have been difficult to detect the difference between their depth preference or the two species did not migrate at exactly the same time and so reached their maximum depths at different times (Pearre, 2003). DVM behaviour is the predominant strategy adopted by the adults, whereas the juveniles did not show significant DVM behaviour. Habitat segregation of the two coexisting *Daphnia* was reinforced by the thermal stratification and the lack of oxygen in the hypolimnion.
The less visible *D. galeata mendotae* was distributed in shallow waters whereas the more conspicuous *D. pulicaria* stayed at the level of the thermocline avoiding both the warm epilimnetic waters (temperature > 22°C) and the anoxic waters (O₂ < 1 mg L⁻¹). Similar habitat segregation has been reported for the same *Daphnia* species coexisting in a hypereutrophic lake (Threlkeld, 1979). By staying in the upper water column at the end of the summer, *D. galeata mendotae* benefited from optimal environmental conditions as shown by higher reproduction and abundance when compared with *D. pulicaria*. In a hypereutrophic lake, the light would be rapidly attenuated, so plankters would not need to go far from the surface to find refuge (Pearre, 2003).

This study, as do other experimental and field studies (Ringelberg *et al.*, 1997; Boersma *et al.*, 1998; Lass and Spaak, 2003) provides strong evidence that refuge behaviour (deeper WMD and DVM) and life history responses (smaller body length, clutch size and SAM) are alternative prominent antipredator defenses against YOY fish (Sakwinska and Dawidowicz, 2005). Our field study indicated that deep habitat selection may be the dominant defense used by the adults of both *Daphnia* species to withstand high negative size-selective predation by small YOY yellow perch. Nonetheless, on 15 July, this response (WMD) was replaced by a decreased SAM, demonstrating the uncoupling of traits within Lake Brome in two coexisting species. Our study also supports the conclusions of Vos *et al.* (2002), who showed using an individual-based modelling approach, that DVM confers a much stronger protection against YOY fish predation than a decreased size at first.
reproduction (SFR). They found that the coupling of DVM behaviour with a decreased SFR was the best scenario to sustain *Daphnia* populations. The alternative use of DVM behaviour alone was almost as efficient as the coupling of behavioural and life history defenses. In contrast, using only life history strategy was the worst scenario with a decline in *Daphnia* populations four times higher. Different phenotypic plasticity in antipredator traits in coexisting prey are considered beneficial in sustaining biodiversity and ecosystem function and in decoupling potential trophic cascades in freshwater pelagic ecosystems (Leibold and Tessier, 1998; Bernot et al., 2004).

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