AUTOREFERAT

1. Imię i Nazwisko

Mirosław Ślusarczyk

2. Posiadane stopnie naukowe

- magister biologii, specjalność biologia środowiskowa, Uniwersytet Warszawski, 1991r., tytuł pracy magisterskiej: „*Wpływ planktonożernej ryby na demografię dominujących gatunków zooplanktonu w jeziorze Wirbel*”
- doktor nauk biologicznych w zakresie biologii, Uniwersytet Warszawski, 1999 r., tytuł pracy doktorskiej „*Diapauza jako mechanizm obrony przed drapieżnictwem u Daphnia magna Strauss*”

3. Informacje o dotychczasowym zatrudnieniu w jednostkach naukowych

- 1991-1999 asystent w Zakładzie Hydrobiologii na Wydziale Biologii Uniwersytetu Warszawskiego,
- 2000-2001 adiunkt kontraktowy w Zakładzie Hydrobiologii, na Wydziale Biologii Uniwersytetu Warszawskiego,
- 2001-2011 adiunkt w Zakładzie Hydrobiologii na Wydziale Biologii Uniwersytetu Warszawskiego.

4a. Osiągnięcia naukowo-badawcze

***Wytłuszczonym drukiem** zaznaczyłem osiągnięcia zawarte w cyklu publikacji „Diapauza jako mechanizm dyspersji w czasie i przestrzeni u skorupiaków planktonowych z rodzaju Daphnia”, stanowiącego podstawę postępowania habilitacyjnego.*

Moje zainteresowania badawcze od początku działalności naukowej koncentrują się wokół ekologii organizmów wodnych, w szczególności: biologii rozwoju, fizjologii, ewolucji strategii życiowych skorupiaków słodkowodnych i ich relacji z abiotycznymi i biotycznymi komponentami zasiedlanych środowisk.

Pierwsze doświadczenia w pracy badawczej nabyłem podczas realizacji pracy magisterskiej zatytułowanej „*Wpływ planktonożernej ryby na demografię dominujących gatunków zooplanktonu w jeziorze Wirbel*” wykonanej pod opieką prof. M. Gliwicza, a wyróżnionej nagrodą drugiego stopnia przez Polskie Towarzystwo Hydrobiologiczne. Wyniki tej pracy opublikowałem kilka lat później w międzynarodowym czasopiśmie naukowym Hydrobiologia (Ślusarczyk 1997). Po obronie pracy magisterskiej w 1991 roku i zatrudnieniu na stanowisku asystenta w Zakładzie Hydrobiologii Uniwersytetu Warszawskiego, uczestniczyłem jako wykonawca pomocniczy w kilku projektach badawczych, testujących skuteczność biologicznych metod poprawy jakości wód jeziornych. W pierwszym z nich (*Wykorzystanie metod biomanipulacji do poprawy jakości wód słodkowodnych*, 1991-1994), podjęliśmy próbę poprawy jakości wód jeziora Wirbel poprzez przebudowę jego struktury troficznej, tj. promocję biomasy glonożernych filtratorów planktonowych, osiągniętą dzięki ograniczeniu presji ryb planktonożernych. W innym projekcie (*Komunikacja chemiczna w systemach słodkowodnych i perspektywy jej zastosowania w gospodarce wodnej i rybackiej*, 1991-1994) testowaliśmy występowanie i rozpowszechnienie międzygatunkowej komunikacji chemicznej wśród zwierząt pelagicznych. W kolejnym projekcie (*Controlling the behavior of planktivorous fish with counterfeit information on risk to predation*, 1995-1996) testowaliśmy możliwość wykorzystania ww. zjawiska do przebudowy struktury troficznej ekosystemów wodnych, w celu poprawy jakości wód jeziornych. Udział w tych projektach skierował moje zainteresowania badawcze na międzygatunkowe relacje drapieżnik–ofiara, którymi zajmuję się do dnia dzisiejszego. Obiektem moich badań laboratoryjnych i terenowych są najczęściej skorupiaki słodkowodne z rodzaju *Daphnia*, stanowiące kluczowy element sieci troficznej w wielu ekosystemach słodkowodnych, będące głównym konsumentem materii pierwotnej i podstawowym pokarmem ryb planktonożernych. Zwierzęta te wykorzystuję w badaniach jako modelowe organizmy ze względu na krótki cykl życiowy (umożliwiający śledzenie zmian ewolucyjnych na przestrzeni pokoleń), specyficzny sposób rozrodu - cykliczną partenogenezę (pozwalającą pozyskać do doświadczeń identyczne genetycznie osobniki), oraz łatwość hodowli.

Podczas realizacji ww. projektów badawczych zainteresowałem się słabo jeszcze poznanym zjawiskiem produkcji form przetrwalnych przez organizmy w okresach zagrożenia ze strony drapieżników. Udokumentowanie tego zjawiska było przedmiotem mojej rozprawy doktorskiej, zatytułowanej *Diapauza jako mechanizm obrony przed drapieżnictwem u Daphnia magna Straus*, realizowanej pod opieką prof. M. Gliwicza. Moje prace laboratoryjne dostarczyły dowodów na występowanie nieopisanego wcześniej mechanizmu wytwarzania form przetrwanych przez potencjalne ofiary w reakcji na obecność w środowisku chemicznych śladów drapieżnictwa ryb planktonożernych (Ślusarczyk 1995). Zjawisko to uznaje się obecnie za jedno z kilku indukowanych mechanizmów obronnych, wykorzystywanych przez skorupiaki słodkowodne w obliczu presji ze strony ryb. W toku dalszych badań ustaliłem, że w indukcji diapauzy u *Daphnia magna* biorą udział co najmniej dwie substancje: kairomony ryb i substancje alarmowe pochodzące z ciał blisko spokrewnionych gatunków ofiar (Ślusarczyk 1999). Substancje te występując pojedynczo nie wywołują tej reakcji lub stymulują do produkcji jaj przetrwalnych niewielki procent samic. Podczas pobytu na stypendium badawczym w instytucie Maxa-Plancka w Ploen w 1997 roku, ustaliłem, że produkcja form przetrwalnych w reakcji na obecność chemicznych śladów drapieżnictwa ryb, nasila się w niekorzystnych, a zanika w korzystnych warunkach pokarmowych (Ślusarczyk 2001). Trzy ostatnie prace eksperymentalne wspólnie z artykułem przeglądowym dotyczącym różnych funkcji oraz mechanizmów indukcji zjawiska spoczynku (Ślusarczyk 1998) weszły w skład mojej rozprawy doktorskiej, którą obroniłem w 1999 r. na Wydziale Biologii Uniwersytetu Warszawskiego.

Kilka miesięcy po obronie pracy doktorskiej zostałem ponownie zatrudniony w macierzystym zakładzie Hydrobiologii UW, korzystając z funduszy grantu KBN *(Diapauza jako mechanizm obrony przed drapieżnictwem* 2000-2003), którego byłem kierownikiem. W latach 2001-2002 przebywałem na stażu podoktorskim w laboratorium limnologicznym kierowanym przez prof. B. Pinell-Alloul na Uniwersytecie Montrealskim. Podczas tego pobytu byłem kierownikiem trzech, oraz wykonawcą pomocniczym w jednym projekcie badawczym finansowanym przez lokalne towarzystwo limnologiczne GRIL (Groupe de Recherche Interuniversitaire en Limnologie*)*. W pierwszym projekcie (*Plasticity of spatio-temporal fish avoidance mechanisms in Daphnia*, 2001) badaliśmy wybór mechanizmów obronnych przez potencjalne ofiary w różnych warunkach środowiskowych. Ustaliliśmy, że behawioralny mechanizm obronny - dobowe migracje pionowe, stanowi preferowany sposób ochrony u *Daphnia catawba* w warunkach zagrożenia ze strony ryb (Ślusarczyk i Pinell-Alloul 2008). W płytkich zbiornikach eksperymentalnych, w których ten mechanizm obronny nie mógł być zastosowany, *Daphnia* angażowały alternatywne, morfologiczne i ontogenetyczne mechanizmy obronne. W drugim projekcie (*Diapause duration and environmental stability*, 2001), porównywaliśmy maksymalną długość spoczynku *Daphnia pulicaria* w zbiornikach wodnych różniących się stabilnością warunków środowiskowych. Nie znaleźliśmy jednak dowodów na istnienie domniemanych różnic u organizmów zasiedlających odmienne środowiska. W kolejnym projekcie, którego byłem kierownikiem, realizowanym w 2002, a kontynuowanym w 2007 roku, (*Ultimate causes of summer diapause of Daphnia pulicaria in the lake Brome)* poszukiwaliśmy przyczyn diapauzy tego skorupiaka w okresie występowania korzystnych warunków abiotycznych. Wyniki naszych badań wskazują na presję drapieżnicą ze strony młodocianego okonia jako prawdopodobną przyczynę występowania tego zjawiska w okresie letnim (Ślusarczyk i in. 2006). W innym projekcie, którego byłem wykonawcą (*Atipredator responses of two coexisting Daphnia species coexisting in a mesotrophic lake, 2002)* badaliśmy sezonową zmienność występowania mechanizmów obronnych u zwierząt planktonowych z rodzaju *Daphnia*. Wyniki naszych badań wskazują, na odmienny sposób indukcji mechanizmów behawioralnych, morfologicznych oraz spoczynku u tych zwierząt oraz na istnienie międzygatunkowych różnic w wyborze mechanizmów obronnych (Gelinais i in 2007, Gelinais i in, 2007b).

Po powrocie z Kanady do macierzystej uczelni kontynuowałem rozpoczęte badania nad mechanizmem indukcji spoczynku przez chemiczne ślady zagrożenia ze strony ryb u skorupiaków planktonowych z rodzaju *Daphnia*. Niestety dalszy postęp badań w tej dziedzinie, utrudniała i nadal utrudnia, nieznajomość składu chemicznego substancji indukujących mechanizmy obronne u tych zwierząt, pomimo wielokrotnych prób ich analizy, prowadzonych w laboratoriach Europejskich i Stanów Zjednoczonych. **Metodyczną trudność kontroli składu i stężenia substancji alarmowych oraz rybich kairomonów w naszych eksperymentach, rozwiązaliśmy doraźnie, proponując oryginalny sposób pozyskiwania tych substancji, wykorzystując odchody ryb planktonożernych (Ślusarczyk i Rygielska 2004). Zaproponowany przez nas sposób pozyskiwania, obróbki i przetrzymywania ekstraktu tych substancji, ułatwia prowadzenie eksperymentów i umożliwia względną kontrolę składu i stężenia czynnych substancji na przestrzeni jednego lub wielu eksperymentów. Do momentu identyfikacji chemicznej substancji indukujących mechanizmy obronne, ekstrakt pozyskiwany w zaproponowany przez nas sposób może stanowić wygodny dla eksperymentatorów erzac substancji chemicznych o zidentyfikowanym składzie.**

W toku dalszych badań ustaliliśmy, że produkcja form spoczynkowych w reakcji na obecność chemicznych śladów zagrożenia ze strony ryb ma plastyczny charakter i zależna jest od kontekstu środowiskowego. Reakcja ta nasila się w warunkach niewielkich szans przeżycia lub wydania potomstwa przez aktywne osobniki, np. w warunkach niewielkiej podaży pokarmu (Ślusarczyk 2001), **niskiej temperatury (Ślusarczyk i Rybicka 2011), czy w sytuacji niemożności zastosowania alternatywnych mechanizmów obronnych (Ślusarczyk i in. 2004), a może zanikać, gdy warunki te ulegną poprawie. W świetle tych badań wytwarzanie form przetrwanych jawi się jako kosztowny mechanizm obronny, który jest uruchamiany przez potencjalne ofiary w sytuacji, gdy mniej kosztowne, aktywne mechanizmy obronne są nieskuteczne lub nie mogą być zastosowane (Ślusarczyk 2004).**

Porównując stopień rozpowszechnienia opisanej wyżej reakcji na obecność chemicznych śladów drapieżnictwa ryb wśród blisko spokrewnionych gatunków *Daphnia* różniących się wielkością ciała, udokumentowaliśmy istnienie międzyklonalnych i międzygatunkowych różnic w reakcji na ww. substancje (Ślusarczyk i in., w druku). Zgodnie z oczekiwaniami, najsilniej na ww. substancje reagowały osobniki największego spośród testowanych gatunków, będące ze względu na swoje duże rozmiary ciała częstą ofiarą ryb planktonożernych. Natomiast najsłabszą reakcję zaobserwowaliśmy wśród osobników

najmniejszego spośród testowanych gatunków, które w naturalnym środowisku, pozostają pod słabą presją ze strony tych drapieżników.

O istotnej roli drapieżnictwa ryb w ewolucji zjawiska spoczynku mogą świadczyć słabo jeszcze udokumentowane przypadki „rezygnacji" z tego mechanizmu w środowiskach bezrybnych. Podczas realizacji projektu badawczego, którego byłem kierownikiem w latach 1996-1998 zatytułowanego „Strategie życiowe *Daphnia pulicaria* w jeziorach o różnej presji selekcyjnej" dokonaliśmy porównania składu zespołu zwierząt planktonowych i właściwości populacji *Daphnia* występujących w dwóch sąsiadujących ze sobą jeziorach tatrzańskich. Wyniki naszych badań potwierdziły kluczową rolę drapieżnictwa ryb planktonożernych w kształtowaniu składu zespołów skorupiaków planktonowych. **Cenniejszego, bo nowego odkrycia, dokonaliśmy analizując demografię populacji wioślarek zasiedlających Czarny Staw pod Rysami, w którym udokumentowaliśmy współistnienie odmiennych strategii przeżycia długiego okresu zimowego, u obligatoryjnie partenogenetycznych osobników dwóch blisko spokrewnionych klonów *Daphnia*. O ile osobniki jednego z klonów zimują w typowy dla daphnide sposób - w postaci jaj przetrwalnych, osobniki drugiego z klonów „rezygnują" z produkcji form przetrwalnych, zimują w postaci aktywnej i cechują się dwukrotnie dłuższym, nieznanym dotychczas w tej grupie organizmów, dwuletnim cyklem życiowym. Uważamy, że ewolucja długowieczności i rezygnacja z produkcji form spoczynkowych była możliwa u tych zwierząt w warunkach braku presji ze strony ryb (**Gliwicz i in. 2001**, Ślusarczyk 2009).**

W trakcie badań tatrzańskich zainteresowaliśmy się masowym występowaniem form przetrwalnych *Daphnia* na powierzchni wody badanych jezior. Rozpowszechnienie, mechanizm oraz ewentualne funkcje tego zjawiska stały się przedmiotem badań w kolejnym projekcie KBN, którego byłem kierownikiem w latach 2004-2008, zatytułowanym „*Mechanizmy dyspersji form przetrwalnych planktonowych wioślarek*". W trakcie prowadzonych badań udokumentowaliśmy występowanie tego zjawiska, we wszystkich badanych akwenach pn-wschodniej Polski. Ustaliliśmy ponadto, że większość występujących na powierzchni wody form przetrwalnych *Daphnia* deponowana jest tam „celowo" przez samice, pomimo znacznych kosztów związanych z tym behawiorem, co wskazuje na istotne funkcje tego zjawiska (Ślusarczyk i Pietrzak 2008). W kolejnym, przygotowanym do druku artykule argumentujemy, że prawdopodobną funkcją tego zjawiska jest wzrost prawdopodobieństwa dyspersji potomstwa pomiędzy zbiornikami (Ślusarczyk, Grabowski, Pietrzak przygotowane do druku).

Swoje spostrzeżenia dotyczące zjawiska spoczynku zawarłem w czterech artykułach koncepcyjno-przeglądowych, których jestem autorem lub współautorem. Pierwszy, najbardziej ogólny, mówi o różnych funkcjach i mechanizmach indukcji zjawiska spoczynku oraz o jego rozpowszechnieniu (Ślusarczyk 1998). Drugi artykuł, porusza kwestie wykorzystania diapauzy przez organizmy do ochrony przed presją ze strony drapieżników (Ślusarczyk 1999). **W trzecim, rozważam korzyści i koszty zaobserwowanej u *Daphnia magna* plastyczności mechanizmu indukcji diapauzy w reakcji na chemiczne ślady zagrożenia ze strony ryb, w różnych warunkach środowiskowych (Ślusarczyk 2004). Czwarty artykuł, którego jestem współautorem, mówi o możliwości wykorzystania form przetrwalnych przez zwierzęta planktonowe do biernej dyspersji pomiędzy akwenami (Pietrzak i Ślusarczyk 2008). Zwracamy w nim uwagę na to, że wykorzystywana powszechnie przez rośliny ww. funkcja spoczynku, może być także wykorzystywana przez zwierzęta osiadłe lub występujące w silnie izolowanych środowiskach, co potwierdzają m. in. wyniki naszych badań.**

4 b. Dokładniejsze omówienie cyklu publikacji stanowiącego podstawę postępowania habilitacyjnego zgodnie z art. 16 ust. 2 ustawy z dnia 14 marca 2003 r. o stopniach naukowych i tytule naukowym oraz o stopniach i tytule w zakresie sztuki (Dz. U. nr 65, poz. 595 ze zm.):

Prace źródłowe:

1. **Ślusarczyk M, Rygielska E. Fish faeces as the primary source of chemical cues inducing fish avoidance diapause in *Daphnia magna*. 2004. Hydrobiologia 526: 231-234.**

Celem badań było ustalenie sposobu uwalniania do środowiska przez planktonożerne ryby substancji chemicznych stymulujących mechanizm spoczynku u skorupiaków planktonowych z rodzaju *Daphnia* oraz opracowanie efektywnej metody pozyskiwania i przechowywania tych substancji. Wyniki przeprowadzonych testów laboratoryjnych wskazują na to, że odchody ryb planktonożernych są prawdopodobnie głównym źródłem ww. substancji w środowisku. Ponadto opracowaliśmy metodę pozyskiwania (z odchodów ryb planktonożernych), ekstrakcji (wodnej) i przechowywania (w stanie zamrożonym) koncentratu ww. substancji. Opisana metoda wykorzystywana jest do dzisiaj w eksperymentach nad indukcją diapauzy oraz innych mechanizmów obronnych skorupiaków planktonowych z rodzaju *Daphnia* w naszym laboratorium.

2. **Ślusarczyk M, Dawidowicz P, Rygielska E. Hide, rest or die: a light-mediated diapause response to the threat of fish predation in *Daphnia magna*. 2005. Freshwater Biology 50: 141-146.**

Podczas badań laboratoryjnych zamierzaliśmy sprawdzić, czy możliwość wykorzystania alternatywnych do diapauzy mechanizmów obronnych może wpływać na „skłonność” potencjalnych ofiar do produkcji form przetrwanych w sytuacji zagrożenia ze strony drapieżnika.

Jednym z najczęściej wykorzystywanych przez zwierzęta pelagiczne (zasiedlające otwartą toń zbiorników wodnych) mechanizmów obronnych przed presją ze strony „drapieżników wzrokowych” jest migracja w ciągu dnia do głębszych, zacienionych stref zbiorników wodnych, gdzie zwierzęta pelagiczne mogą pozostawać względnie bezpieczne pomimo obecności w środowisku aktywnych pokarmowo drapieżników.

W eksperymencie laboratoryjnym eksponowaliśmy zwierzęta pelagiczne (*Daphnia magna*) na chemiczne ślady drapieżnictwa ryb, w warunkach różnego natężenia światła, determinującego w warunkach naturalnych stopień realnego zagrożenia ze strony ryb planktonożernych.

Wraz ze wzrostem natężenia światła zanotowaliśmy wzrost udziału osobników wytwarzających formy przetrwalne. W sytuacji nieobecności chemicznych śladów drapieżnictwa, proporcja osobników wytwarzających formy przetrwalne była bliska zeru, w obrębie testowanych warunków świetlnych.

Wyniki eksperymentu wskazują na to, że dostęp do refugiów może wpływać na „skłonność” potencjalnych ofiar do produkcji form przetrwanych w okresie zagrożenia ze strony „drapieżników wzrokowych”.

3. **Ślusarczyk M, Rybicka B. Role of temperature in diapause response to fish kairomones in crustacean *Daphnia*. 2011. Journal of Insect Physiology 57: 676-680.**

W eksperymencie laboratoryjnym testowaliśmy wpływ warunków termicznych (bliskich wartościom optymalnym) na skłonność do produkcji form przetrwanych przez potencjalne ofiary w sytuacji zagrożenia ze strony drapieżnika.

Wraz ze wzrostem temperatury zanotowaliśmy spadek proporcji zwierząt eksperymentalnych (*Daphnia magna*) wytwarzających formy przetrwane w warunkach symulowanego (przy użyciu chemicznych śladów presji drapieżniczej) zagrożenia ze strony drapieżników (ryb planktonożernych). W wariantach pozbawionych chemicznych śladów drapieżnictwa, udział osobników wytwarzających formy przetrwalne pozostawał na niskim poziomie, niezależnie od testowanych warunków termicznych. Wyniki eksperymentu interpretujemy jako przejaw strategii maksymalizacji sukcesu rozrodczego organizmów w warunkach presji drapieżniczej. W niskiej temperaturze, w obliczu presji ze strony ryb, produkcja form przetrwalnych (zapewniająca niskie, ale względne stałe korzyści w różnych warunkach termicznych) może być bardziej opłacalna niż spowolniony rozwój i rozród w stanie aktywnym. W wyższej temperaturze szybszy rozwój i rozród aktywnych osobników może zapewnić większe korzyści niż produkcja form przetrwalnych.

4. **Ślusarczyk M, Ochocka A, Cichocka D. The prevalence of diapause response to risk of size selective predation in small and large bodied prey species. 2012. Aquatic Ecology. 00: 000-000. DOI: 10.1007/s10452-011-9376-6**

W eksperymencie laboratoryjnym porównaliśmy częstość wytwarzania form przetrwanych u trzech blisko spokrewnionych gatunków *Daphnia* (*D. magna*, *D. pulicaria* i *D. longispina*) różniących się wielkością ciała, w warunkach symulowanej presji drapieżniczej. Najwyższy udział osobników wytwarzających formy przetrwalne zanotowaliśmy u osobników największego spośród testowanych gatunków, poddanych w warunkach naturalnych najsilniejszej presji ze strony ryb planktonożernych. Najniższą częstość produkcji form przetrwanych zanotowaliśmy u najmniejszego spośród testowanych gatunków, najmniej chętnie spożywanego przez ryby.

Wyniki badań wskazują na to, że wytwarzania form przetrwanych można spodziewać się przede wszystkim u gatunków organizmów, w największym stopniu narażonych na presję ze strony drapieżników.

5. **Ślusarczyk M, Pietrzak B. To sink or float: the fate of dormant offspring is determined by maternal behaviour in *Daphnia*. 2008. Freshwater Biology 53: 569–576.**

W artykule prezentujemy wyniki badań terenowych oraz laboratoryjnych dotyczących zjawiska występowania form przetrwanych skorupiaków planktonowych z rodzaju *Daphnia* na powierzchni wód.

W pracy udokumentowaliśmy:

1) powszechne występowanie form przetrwanych kilku gatunków *Daphnia* na powierzchni wód we wszystkich (kilkunastu) badanych akwenach (stawach i jeziorach),

2) behawioralne podłoże tego zjawiska – większość uwalnianych w warunkach laboratoryjnych jaj przetrwanych deponowana była na powierzchni wody przez samice pomimo ryzyka związanego z tym behawiorem,

3) istnienie różnic międzygatunkowych w częstości występowania tego zjawiska u zwierząt z rodzaju *Daphnia.*

Naszym zdaniem funkcją aktywnej depozycji form przetrwalnych na powierzchni wód przez skorupiaki planktonowe z rodzaju *Daphnia* jest wzrost szans biernej dyspersji potomstwa do sąsiednich środowisk przez ożywione i nieożywione wektory, operujące na powierzchni wód.

6. **Ślusarczyk M. Extended lifespan traded for diapause in *Daphnia*. 2009. Freshwater Biology 54: 2252-2262.**

W trakcie trzyletnich badań terenowych analizowałem różne strategie przetrwania długiego okresu niesprzyjających warunków zimowych w dwóch populacjach blisko spokrewnionych, obligatoryjnie partenogenetycznych gatunków *Daphnia*, występujących w wysokogórskim jeziorze - Czarnym Stawie Pod Rysami. O ile osobniki jednego z klonów zimują w typowy dla daphnidae sposób - w postaci jaj przetrwanych, osobniki drugiego z klonów „rezygnują” z produkcji form przetrwalnych, zimują w postaci aktywnej i cechują się dwukrotnie dłuższym, nieznanym dotychczas w tej grupie organizmów, dwuletnim cyklem życiowym. Obie współwystępujące w tym zbiorniku strategie życiowe, umożliwiają rozród i rozwój potomstwa, w krótkim sezonie korzystnych warunków pokarmowych na przełomie wiosny i lata. W artykule omawiam możliwe przyczyny „rezygnacji” w tym ekstremalnym środowisku z rozrodu płciowego przez oba gatunki oraz z produkcji form przetrwanych przez jeden z nich.

Prace koncepcyjno przeglądowe:

7. **Ślusarczyk M. Environmental plasticity of fish avoidance diapause response in *Daphnia magna*. 2004. Journal of Limnology 63: 70-74.**

W tym koncepcyjnym artykule podsumowuję wyniki własnych prac eksperymentalnych nad plastycznością fenotypową mechanizmu indukcji diapauzy w różnych warunkach środowiskowych (świetlnych, termicznych i pokarmowych) determinujących szanse przeżycia i wydania potomstwa przez aktywne osobniki w obliczu presji ze strony drapieżnika.
Wyniki naszych badań wskazują na to, że diapauza jest kosztownym mechanizmem obronnym, uruchamianym przez potencjalne ofiary w sytuacji, gdy szansa na rozwój i rozród w stanie aktywnym spada poniżej poziomu korzyści jakie zapewnia produkcja form przetrwanych.

8. **Pietrzak B, Ślusarczyk M. The fate of ephippia - *Daphnia* dispersal in time and space. 2006. Polish Journal of Ecology 54: 709-714.**

W tym koncepcyjno-przeglądowym artykule analizujemy dwie funkcje, jakie może pełnić zjawisko spoczynku w życiu skorupiaków planktonowych: umożliwienie dyspersji ich genomu w czasie lub przestrzeni. W tym artykule prezentujemy oryginalną hipotezę, która jest przedmiotem naszych dalszych badań, wg której występowanie na powierzchni wód form przetrwanych skorupiaków planktonowych z rodzaju *Daphnia* nie jest zjawiskiem przypadkowym, a efektem „celowego" działania osobników produkujących formy przetrwalne, zmierzającego do zwiększenia prawdopodobieństwa ich biernej dyspersji do sąsiednich środowisk, przez ożywione i nieożywione wektory dyspersji.

Inne, własne publikacje cytowane w autoreferacie:

Ślusarczyk M. Predation-induced diapause in *Daphnia*. 1995. Ecology, 76, 1008-1013.

Ślusarczyk M. Impact of fish predation on a small-bodied cladoceran: limitation or stimulation? 1997. Hydrobiologia 342/343: 215-221.

Ślusarczyk M. Diapauza jako strategia przetrwania (Diapause as a strategy for survival). 1998. Wiadomości Ekologiczne 44: 279-303.

Ślusarczyk M. Predator induced diapause in *Daphnia magna* may require two chemical cues. 1999. Oecologia 119: 159–165.

Ślusarczyk M. Diapauza jako obrona przed drapieżnictwem (Diapause as a predatory avoidance adaptation). 1999. Kosmos 48: 465-476.

Ślusarczyk M. Food threshold for diapause in *Daphnia* under the threat of fish predation. 2001. Ecology 82: 1089-1096.

Ślusarczyk M, Pinel-Alloul B, Gelinas M. On ultimate reasons for summer diapause of *Daphnia* in a permanent lake. 2006. Verh. Internat. Verein. Limnol. 29: 1440-1442.

Gélinas M, Pinel-Alloul B, Ślusarczyk M. Alternative antipredator responses of two coexisting *Daphnia* species to negative size selection by YOY perch. 2007. Journal of Plankton Research 29: 775-789.

Ślusarczyk M, Pinell-Alloul B. Depth selection and life history strategies as mutually exclusive responses to risk of fish predation in *Daphnia*. 2010. Hydrobiologia 643: 33–41.

Ślusarczyk M, Grabowski T, Pietrzak B. Temporal surface banks of *Daphnia* ephippia in deep and shallow lakes. W przygotowaniu.

ACHIEVEMENTS IN RESEARCH ACTIVITIES

1. First and family name

Mirosław Ślusarczyk

2. Scientific titles achieved

- Master of Science in biology, University of Warsaw, 1991, after defending the thesis „*Impact of planktivorous fish on demography of dominant zooplanktonic species in the Lake Wirbel*"
- Phylosophy doctor in biology, University of Warsaw, 1999, after defending the thesis „*Diapause as a predator avoidance adaptation in Daphnia magna Strauss*"

3. Scientific positions held

- 1991-1999 junior assistant at the Department of Hydrobiology, Faculty of Biology, University of Warsaw
- 2000-2001 contracted professor assistant at the Department of Hydrobiology, Faculty of Biology, University of Warsaw
- 2001-2011 contracted professor assistant at the Department of Hydrobiology, Faculty of Biology, University of Warsaw

4a. Scientific achievements

***In bold** I have marked scientific achievements included in a series of publications concerning "Diapause as a mechanism of temporal and spatial dispersal in planktonic crustaceans of the genus Daphnia", evaluated in the habilitation.*

From the beginning of my research activities I am interested in ecology of aquatic organisms, more specifically in developmental biology, physiology, evolution of life strategies of freshwater crustaceans and their relation with the abiotic and biotic components of inhabited environments.
I started my scientific activity by conducting MSc experimental study entitled "*Effect of planktivorous fish on the demography of dominant zooplankton species in Lake Wirbel,*" performed under the supervision of prof. M. Gliwicz. This thesis achieved the second grade reward from the Polish Hydrobiological Society. I published the results of this study a few years later in the international scientific journal Hydrobiologia (Ślusarczyk 1997). After defending MSc thesis I was employed as an assistant in the Hydrobiology Department, University of Warsaw in 1991. At the beginning of my academic career I participated in a few research projects developing biological

methods of lake water quality improvement. In the first study (*Utilisation of biomanipulation methods in lake water quality improvement*, 1991-1994), we tried to improve the water quality of Lake Wirbel by shaping its trophic structure, more specifically by promotion of phytophagous planktonic crustaceans due to reduction of planktivorous fish pressure. In another project (*Chemical communication in freshwater systems and prospects for its use in aquaculture and fisheries*, 1991-1994) we tested the prevalence of interspecific chemical communication among pelagic animals. In another project (*Controlling the behavior of planktivorous fish with counterfeit information on risk to predation*, 1995-1996), we tested the possibility of utilization of chemical communication for improvement of lake water quality due to imposed changes in the trophic structure of aquatic ecosystems. Participation in these projects directed my interest on interspecific interactions between prey and predator, which I deal with to this day. Freshwater crustaceans of the genus *Daphnia* are the most frequently used organisms in my laboratory and field studies. They are, the key element of the food web in many freshwater ecosystems where they are the major consumer of primary matter and the basic food of planktivorous fish. These animals are used as model organisms in many laboratories, due to the specific way of reproduction - cyclic parthenogenesis (which let form genetically identical individuals), short life cycle (that allows trace evolutionary changes over many generations), and ease of breeding.

Soon I paid attention in the poorly explored phenomenon of the production of diapausing forms by potential prey in periods of high danger from predators. The research of this phenomenon was the key subject of my doctoral dissertation, entitled "*Diapause as a mechanism of defense against predation in Daphnia magna Straus*", that I did under the supervision of prof. M. Gliwicz. The results of my laboratory work documented the existence of a mechanism of formation of diapausing forms by potential prey in response to the presence of chemical cues associated with fish predation (Ślusarczyk 1995). Presently this phenomenon is considered as one of a few inducible defenses used by freshwater crustaceans confronted with the risk of fish predation. In my further study I found out that at least two different cues are involved in the diapause induction in *Daphnia*: fish kairomones and alarm substances derived from injured conspecific prey (Ślusarczyk 1999). During my research fellowship at the Max-Planck Institute in Ploen in 1997, I found out that the production of diapausing forms in response to the presence of chemical cues of fish predation, intensified at poor while diminished at favorable food conditions (Ślusarczyk 2001). Results of these three experimental studies together with a review article concerning various functions and mechanisms of diapause induction (Ślusarczyk 1998) constituted my PhD thesis which I defended in 1999.

A few months after the PhD defense I was employed again in the Hydrobiology Department of University of Warsaw, supported by the KBN grant money of the project managed by myself

(*Diapause as a defense mechanism against predation* 2000-2003). In 2001-2002 I was employed as postdoctoral fellow in the laboratory of Prof. B. Pinell-Alloul at the University of Montreal. During this time I coordinated three, and was a contractor of another research projects funded by the GRIL (Groupe de Recherche en Interuniversitaire Limnology) local limnological scientific society in Montreal. In the first project (*Plasticity of Spatio-Temporal Mechanisms of fish avoidance in Daphnia*, 2001) we examined the choice of defense mechanisms by potential prey in a variety of environmental conditions. We found that behavioral mechanism - the diurnal vertical migrations, is the preferred defense of *D. catawba* exposed to the threat of fish predation (Ślusarczyk and Pinell-Alloul 2008). In shallow water, where the preferred defense may not have been used by *Daphnia* it employed some life history defenses instead (relative diminution of body size that allows reduction of predatory pressure from the size selective fish). In the second project (*Diapause duration and environmental stability*, 2001), we compared the persistence in diapause of *D. pulicaria* in various reservoirs differing in range of environmental stability. However, we did not find expected differences between variable and invariable lakes. In another coordinated project, conducted in 2002 and continued in 2007, (*Ultimate causes of summer diapause of Daphnia pulicaria in the lake Brome*), we were looking for ultimate reasons of seasonal diapause during periods of favorable abiotic conditions. Our results indicate predatory pressure from juvenile perch as the most likely reason of this phenomenon in summer (Ślusarczyk et al. 2006). In another project, that I was a contractor (*Antipredator responses of two coexisting Daphnia species in a mesotrophic lake*, 2002) we examined the seasonal variation in prevalence of defense mechanisms in planktonic animals of the genus *Daphnia*. Our results evidenced different way of induction of behavioral, morphological and life-history responses in planktonic cladocerans and the existence of interspecific differences in the choice of the preferred defense (Gelinais et al 2007, Gelinais et al, 2007b).

After returning from Canada to my alma mater I continued suspended research on the mechanism of induction of dormancy by chemical cues of fish predation in planktonic crustaceans of the genus *Daphnia*. **The further progress of the research in this area was hindered by ignorance of the chemical composition of the inductive cues, despite repeated attempts at analysis, conducted in the laboratories in Europe and United States. We solved the spot, proposing an original way of gathering these substances, using fish faeces (Ślusarczyk and Rygielska 2004). This method of collection, processing and storage of the natural inductive cues, facilitated further research and allowed relative control of the composition and concentrations of the active substance within and between experiments. Until the chemical composition of these compounds is known, our method of their collection and storage may be offer convenient solution to this problem.**

In the following studies, we found that the decision of potential prey to produce diapausing forms in response to the presence of chemical cues of fish predation may depend on the environmental context. The prevalence of this reaction seems to increase at lower chances of survival or reproduction of active forms deteriorated by low food supply (Ślusarczyk 2001), low temperature (Ślusarczyk and Rybicka 2011), or when alternative active defenses might not be used (Ślusarczyk et al. 2004). In light of these studies diapause reaction seems to be a costly response employed by potential prey when less costly, active defenses appear ineffective or can not be used (Ślusarczyk 2004).

By comparison of the prevalence of diapause reaction to chemical cues on predation among closely related species of *Daphnia* that differed in body size, we documented the existence of interspecific and intraspecific differences in response to the tested cues of predation (Ślusarczyk et al, in press). As expected, the highest prevalence of diapause response we observed in *D. magna*, which due to their large body size is most eagerly eaten by fish. The lowest prevalence we observed among the smallest of the tested species - *D. longispina* which in natural conditions, remain under low pressure from these predators.

The important role of fish predation in the selection of diapause phenomenon in *Daphnia* may indicate poorly explored cases of abandoning of this mechanism in fishless habitats. During the research project, coordinated by me in 1996-1998, entitled "*Life strategies of Daphnia pulicaria in lakes of varying selective pressure*" we made a comparison of the community composition and properties of planktonic animals inhabiting two adjacent lakes inhabited and not inhabited by fish in the Tatra Mountains. Our results confirmed the key role of fish predation in shaping the composition of community assemblage of planktonic crustaceans (Gliwicz et al. 2001). **More detailed analysis of population structure of *Daphnia* inhabiting one of the studied lake - Czarny Staw documented the coexistence of two survival strategies during the long winter period, in two closely related obligatory parthenogenetic *Daphnia* clones of the pulex complex. While individuals of one of the clones overwinter in typical way for daphnidae as diapausing eggs, specimens of the other clones "resigned" from the production of diapausing eggs, overwintered in active form, extended their lifespan twofold and reproduced next spring in the short period of relatively favourable food conditions. We claim that the evolution of longevity and the abandoning of the resting egg formation was possible in the absence of pressure from fish** (Gliwicz et al. 2001, **Ślusarczyk 2009**).

During this project we paid our attention to the mass occurrence of diapausing eggs of *Daphnia* at the water surface. The prevalence, mechanism and functions of this phenomenon became the research topic of the next project, which I coordinated in 2004-2008, entitled

"*Mechanisms of dispersion of planktonic cladocerans*." In the course of this study we documented wide prevalence of this phenomenon, in all studied waterbodies in north-eastern Poland. We found out that the majority of diapausing forms of *Daphnia* that appears at the water surface is deposited there "on purpose" by the mothers, despite substantial costs associated with this behavior, which indicates some important function of this phenomenon (Ślusarczyk and Pietrzak 2008). In the following paper, we argue that the most likely function of this phenomenon is to increase the chances of offspring dispersion between waterbodies (Ślusarczyk, Pietrzak, Grabowski in preparation).

My knowledge and suppositions concerning the diapause phenomenon I included in four conceptual articles. The first, most general one, describes the different functions and mechanisms of induction of this phenomenon (Ślusarczyk 1998). The second article, considers diapause as a way of protection against pressure from predators (Ślusarczyk 1999). **In the third article, I discuss costs and benefits of environmentally driven plasticity of the kairomone induced diapause response (Ślusarczyk 2004). The fourth article, which I am co-author, regards possible use of resting forms for passive dispersion between waterbodies by planktonic animals (Pietrzak and Ślusarczyk 2008). We claim that utilization of diapausing forms for passive dispersal may be the common phenomenon not only in plants but also in sessile animals and ones inhabiting isolated sites as evidenced by our study.**

4b. More detailed description of a series of publications "*Diapause as a mechanism of temporal and spatial dispersal in planktonic crustaceans of the genus Daphnia",* **presented as the basis for habilitation:**

1. **Ślusarczyk M, Rygielska E. Fish faeces as the primary source of chemical cues inducing fish avoidance diapause in *Daphnia magna*. 2004. Hydrobiologia 526: 231-234.**

In laboratory tests we aimed to test potential pathways of excretion of the chemical stimuli that induce resting-egg formation in *D. magna* and to find an effective way of collecting these chemical cues. The results indicate that fish faeces are the prevailing source of the chemical cues that induce resting-egg production in *D. magna*. The ease of collection and the possibility of storing it in a frozen state make it a convenient cue for inducing diapause response in *Daphnia*. The proposed method has been used for years in experimental research on induction of diapause and other defense mechanisms of planktonic crustaceans of the genus *Daphnia* in our laboratory.

2. **Ślusarczyk M, Dawidowicz P, Rygielska E. Hide, rest or die: a light-mediated diapause response to the threat of fish predation in *Daphnia magna*. 2005. Freshwater Biology 50: 141-146.**

In a laboratory experiment, a diapause response of *Daphnia magna* to a simulated threat of fish predation was tested at various light intensities, which under natural conditions determine potential vulnerability of *Daphnia* to visual planktivorous fish.

Under moderate light intensity that allows effective predation by fish, the proportion of females producing dormant eggs was significantly higher than under dim light conditions that are not favourable for visual detection of prey. Production of dormant eggs was not observed in complete darkness or in treatments missing fish kairomones, irrespective of tested light conditions.

The observed phenomenon is interpreted as a flexible response of prey to the conditional risk of predation assessed by *Daphnia* according to the presence of fish-derived cues on the one hand and the presence of dark refugia on the other. Irrespective of the presence of fish kairomones, *Daphnia* may not produce resting eggs as long as a safe, dark, bottom zone is accessible.

3. **Ślusarczyk M, Rybicka B. Role of temperature in diapause response to fish kairomones in crustacean *Daphnia*. 2011. Journal of Insect Physiology 57: 676-680.**

The effect of non-lethal thermal conditions on the incidence of diapause response to a simulated threat of fish predation in *Daphnia magna* was tested in a laboratory experiment. At higher temperatures, with consequently faster development and more intense reproduction, a smaller proportion of *Daphnia* produced protective, diapausing eggs when exposed to fish kairomones than was the case in colder conditions. In environments free of fish kairomones, the production of diapausing eggs was not observed in any of the tested temperatures.

These findings in *Daphnia* are explained in line with strategies for the maximisation of reproductive success under predation risk at various thermal conditions. While production of well protected diapausing eggs (which assures low yet stable gains at various environmental conditions) may appear a more beneficial life history strategy at lower temperatures – where the chances of survival of fish predation by active offspring are low – higher temperatures could permit intensive reproduction that surpasses numerical losses of active descendants due to predation, making diapause less rewarding option.

4. **Ślusarczyk M, Ochocka A, Cichocka D. The prevalence of diapause response to risk of size selective predation in small and large bodied prey species. 2012. Aquatic Ecology. 00: 000-000. DOI: 10.1007/s10452-011-9376-6**

The prevalence of diapause response to the simulated threat of fish predation was compared in three species of *Daphnia* (*D. magna, D. pulicaria and D. longispina*), which due to their different body size vary in vulnerability to fish predation pressure in natural conditions. Higher incidence of diapause response was presumed in the larger bodied species which due to their higher conspicuousness and higher energetic content experience the greatest size-selective pressure from visually foraging fish. Small bodied species were expected to utilize less costly yet less effective active defences e.g. lower conspicuousness achieved due to slower body growth when facing risk of size selective predation.

Proportions of females forming diapausing structures were compared in experimental beakers containing or not containing fish kairomones (chemical cues of fish predation) in a few clonal lineages of 3 species of *Daphnia* derived from a single lake inhabited by fish.

The highest incidence of diapause response to fish kairomones was observed in D. *magna* (when measured both as proportion of sensitive individuals and as proportion of inductive clones), the largest of the tested *Daphnia* species. The lowest proportion of individuals and clones that employed diapause was reported in the smallest tested species, D. longispina.

The results of this study support the assumption of higher prevalence of diapause response to the threat of selective predation in larger and more vulnerable prey species.

5. **Ślusarczyk M, Pietrzak B. To sink or float: the fate of dormant offspring is determined by maternal behaviour in *Daphnia*. 2008. Freshwater Biology 53: 569–576.**

As the ephippia (chitinous shells enclosing diapausing eggs) of pelagic crustaceans of the genus *Daphnia* have been occasionally reported to float at the water surface, we considered that this might be an adaptation promoting their passive dispersal. We investigated the mechanisms by which ephippia appear at the water surface.

While field surveys revealed that floating *Daphnia* ephippia are often numerous in various freshwater habitats, laboratory tests showed that newly formed ephippia are not buoyant initially. Once transferred to the surface by whatever means, however, they may remain there due either to surface tension or gas absorption.

Video recordings showed that all ephippia at the water surface in laboratory vessels were shed there by ephippial females when moulting (despite the attendant risk of exposure to UV radiation). This implies that the moulting behaviour of female *Daphnia* may determine the fate of their dormant offspring, predetermining whether they remain in the natal environment (when the ephippium is released into the water column) or disperse (when it is deposited at the water surface).

Our findings reveal a potential mechanism underlying the high dispersal capacity of freshwater cladocerans inhabiting island-like aquatic habitats.

6. **Ślusarczyk M. Extended lifespan traded for diapause in *Daphnia*. 2009. Freshwater Biology 54: 2252-2262.**

I present here results of a 3-year field study conducted in a deep ultraoligotrophic fish-free alpine-type lake (Czarny Staw in the Tatra mountains in southern Poland) that revealed the coexistence of two closely related asexual lineages of *Daphnia* of the pulex complex, which differ in body colour (transparent versus orange) and in their strategies for surviving long winters.

The 'transparent' clone of European origin exhibits an ephemeral lifestyle. It completes its life cycle within a single season, forming two generations of active specimens during the short summer and producing diapausing eggs late in the season. Transparent individuals live no longer than 5–6 months in this cold lake and survive winter exclusively in the form of diapausing eggs.

Individuals of the 'orange' lineage, which are closely related to eastern Nearctic *Daphnia* pulicaria, exhibit a biennial lifestyle unique to this genus. They do not form diapausing eggs or produce them only occasionally. Instead, they are active throughout the year and live for up to 13–14 months. Reproduction is postponed until the second year of life when food conditions have improved. Rich lipid reserves accumulated in the first season fuel them during the long winter and permit early reproduction the following spring.

I discuss thereafter possible reasons for the evolution of obligatory parthenogenesis and long lifespan in *Daphnia*.

Review and conceptual articles:

7. **Ślusarczyk M. Environmental plasticity of fish avoidance diapause response in *Daphnia magna.* 2004. Journal of Limnology 63: 70-74.**

Our experimental evidences discussed in this mini-review indicate that the reaction of D. *magna* to chemical signals of fish predation could be conditional and determined by key environmental conditions, which in nature affect relative gains of activity vs. dormancy. At high risk of fish predation, the decision of *Daphnia* to produce resting eggs was disfavored by high food concentration. This reaction was claimed adaptive since high food allows for higher reproductive rates and better survival of offspring.

All this may assure higher benefits due to activity despite some risk of predation (once predation pressure is not fatal to all active descendants) and disfavor resting eggs production. Moreover, at moderate food conditions the decision of *Daphnia* to pro-duce resting eggs was disfavored by the availability of a dark refuge from fish visual predators and thus likely lowering the risk of being preyed upon. Furthermore, when food was at a moderate level and a dark refuge was not present the decision of *Daphnia* to produce resting eggs was favoured by low water temperature. This could be explained as an adaptive reaction again, once low water temperature (due to its effect on a rate of metabolism) should have affected the gains derived from active life and reproduction more seriously than ones of inactive stages. The evidence presented here indicates that a responsive diapause allows D. *magna* to maximize reproductive output by taking advantage of opportunities presented by an unpredictable environment.

8. **Pietrzak B, Ślusarczyk M. The fate of ephippia - *Daphnia* dispersal in time and space. 2006. Polish Journal of Ecology 54: 709-714.**

In this conceptual article we consider two functions that diapause may play: temporal and spatial dispersal. While dormancy and dispersal of ephippia have been well studied, initial factors determining the fate of the ephippia have remained unknown.

We first consider the fate of an ephippium that sinks to the bottom of the water body, where it enriches the egg bank present in the sediments. We then discuss the potential and evidence for dispersal of ephippia, which is probably facilitated by their flotation on the water surface. Finally, we concentrate on the moment of the release of the ephippium showing a likely mechanism that determines its further fate – temporal or spatial dispersal – and we demonstrate it to be accomplished through maternal behaviour.

Other publications cited:

Ślusarczyk M. Predation-induced diapause in *Daphnia*. 1995. Ecology 76, 1008-1013.

Ślusarczyk M. Impact of fish predation on a small-bodied cladoceran:limitation or stimulation? 1997. Hydrobiologia 342/343: 215-221.

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Ślusarczyk M. Predator induced diapause in *Daphnia magna* may require two chemical cues. 1999. Oecologia 119: 159–165.

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Ślusarczyk M, Pinel-Alloul B, Gelinas M. On ultimate reasons for summer diapause of *Daphnia* in a permanent lake. 2006. Verh. Internat. Verein. Limnol. 29: 1440-1442.

Gélinas M, Pinel-Alloul B, Ślusarczyk M. Alternative antipredator responses of two coexisting *Daphnia* species to negative size selection by YOY perch. 2007. Journal of Plankton Research 29: 775-789.

Ślusarczyk M, Pinell-Alloul B. Depth selection and life history strategies as mutually exclusive responses to risk of fish predation in *Daphnia.* 2010. Hydrobiologia 643: 33–41.

Ślusarczyk M, Grabowski T, Pietrzak B. Temporal surface banks of *Daphnia* ephippia in deep and shallow lakes. In preparation.

Wykaz własnych oraz współautorskich prac naukowych wraz z wartościami Impact Factor czasopism, w których się ukazały. Wartości IF_{2010} wyliczone zostały na podstawie bazy Journal Citation Reports 2010.

CYKL PUBLIKACJI PO DOKTORACIE, ZGŁOSZONY JAKO PODSTAWA POSTĘPOWANIA HABILITACYJNEGO:

2012 **Ślusarczyk M.**, Ochocka A., Cichocka D. The prevalence of diapause response to risk of size selective predation in small and large bodied prey species. Aquatic Ecology 00: 000-000. DOI: 10.1007/s10452-011-9376-6. IF_{2010} = 1,4

2011 **Ślusarczyk M.,** Rybicka B. Role of temperature in diapause response to fish kairomones in crustacean *Daphnia*. Journal of Insect Physiology. IF_{2010} = 2,3

2009 **Ślusarczyk M**. Extended lifespan traded for diapause in *Daphnia*. Freshwater Biology 54: 2252-2262. IF_{2010} = 3,1

2008 **Ślusarczyk M.,** Pietrzak B. To sink or float: the fate of dormant offspring is determined by maternal behaviour in *Daphnia.* Freshwater Biology 53: 569–576. IF_{2010} = 3,1

2006 Pietrzak B., **Ślusarczyk M**. The fate of ephippia - *Daphnia* dispersal in time and space. Polish Journal of Ecology 54: 709-714. IF_{2010} = 0,5

2005 **Ślusarczyk M.,** Dawidowicz P., Rygielska E. Hide, rest or die: a light-mediated diapause response to the threat of fish predation in *Daphnia magna*. Freshwater Biology 50: 141-146. IF_{2010} = 3,1

2004 **Ślusarczyk M**. Environmental plasticity of fish avoidance diapause response in *Daphnia magna.* Journal of Limnology 63: 70-74. IF_{2010} = 1,1

2004 **Ślusarczyk M.**, Rygielska E. Fish faeces as the primary source of chemical cues inducing fish avoidance diapause in *Daphnia magna*. Hydrobiologia 526: 231-234. IF_{2010} = 2,0

INNE PUBLIKACJE, KTÓRE UKAZAŁY SIĘ PO DOKTORACIE

2010 **Ślusarczyk M.,** Pinel-Alloul B. Depth selection and life history strategies as mutually exclusive responses to risk of fish predation in *Daphnia*. Hydrobiologia 643:33–41. IF_{2010} = 2,0

Własny udział w publikacji artykułu oceniam na 70%. Polegał on na zaplanowaniu badań, zbieraniu i analizie materiału oraz pisaniu artykułu.

2007 Gélinas M., Pinel-Alloul B., **Ślusarczyk M.** Formation of morphological defences in response to YOY perch and invertebrate predation in two *Daphnia* species coexisting in a mesotrophic lake. Hydrobiologia 594:175–185. IF_{2010} = 2,0
Własny udział w publikacji artykułu oceniam na 20%. Polegał on na planowaniu badań, zbieraniu i analizie materiału oraz korekcie manuskryptu

2007 Gélinas M., Pinel-Alloul B., **Ślusarczyk M.** Alternative antipredator responses of two coexisting *Daphnia* species to negative size selection by YOY perch. J. Plankton Res. 29: 775-789. IF_{2010} = 1,7
Własny udział w publikacji artykułu oceniam na 20%. Polegał on na planowaniu badań, zbieraniu i analizie materiału oraz korekcie manuskryptu.

2006 **Ślusarczyk M.** Pinel-Alloul, Gélinas M. On ultimate reasons for summer diapause of *Daphnia* in a permanent lake. Verh. Internat. Verein. Limnol. 29: 1440-1442. Czasopismo nie uwzglednione w indeksie cytowań JCR.
Własny udział w publikacji artykułu oceniam na 60%. Polegał on na zaplanowaniu badań, zbieraniu i analizie materiału oraz pisaniu artykułu.

2001 Gliwicz Z.M., Ślusarczyk A. **Ślusarczyk M.** - Life-history synchronization in a long-lifespan single-cohort *Daphnia* population of an alpine lake free of fish – Oecologia 128: 368-378. IF_{2009} = 3,5
Własny udział w publikacji artykułu oceniam na 15%. Polegał on na planowaniu badań, zbieraniu i analizie materiału oraz korekcie manuskryptu.

1999 **Ślusarczyk M.** Diapauza jako obrona przed drapieżnictwem (Diapause as a predatory avoidance adaptation). Kosmos 48: 465-476. Czasopismo nie uwzględnione w indeksie cytowań JCR

PUBLIKACJE POWSTAŁE, NA PODSTAWIE MATERIAŁÓW Z PRACY DOKTORSKIEJ

2001 **Ślusarczyk M**. Food threshold for diapause in *Daphnia* under the threat of fish predation. Ecology 82: 1089-1096. IF_{2010} = 5,1

1999 **Ślusarczyk M.** Predator induced diapause in *Daphnia magna* may require two chemical cues. Oecologia 119: 159–165. IF_{2010} = 3,5

1998 **Ślusarczyk, M.** Diapauza jako strategia przetrwania (Diapause as a strategy for survival). Wiadomości Ekologiczne 44: 279-303. Czasopismo nie uwzglednione w indeksie cytowań JCR

1995 **Ślusarczyk, M.** Predation-induced diapause in *Daphnia.* Ecology, 76, 1008-1013. $IF_{2010} = 5,1$

INNE PUBLIKACJE KTÓRE UKAZAŁY SIĘ PRZED DOKTORATEM

1997 **Ślusarczyk M.** Impact of fish predation on a small-bodied cladoceran: limitation or stimulation? Hydrobiologia 342/343: 215-221. $IF_{2010} = 2,0$

Liczba cytowań ww. publikacji według bazy Web of Science z dnia 12.11.2011 = 219

Indeks Hirscha ww. publikacji według bazy Web of Science z dnia 12.11.2011 = 7

List of publications with Impact Factor of journals they were published in, according to the Journal Citation Reports 2010.

Articles included in the series "*Diapause as a mechanism of temporal and spatial dispersal in planktonic crustaceans of the genus Daphnia*" published after PhD dissertation:

2012	**Ślusarczyk M.**, Ochocka A., Cichocka D. The prevalence of diapause response to risk of size selective predation in small and large bodied prey species. Aquatic Ecology 00: 000-000. DOI: 10.1007/s10452-011-9376-6. IF_{2010} = 1.4
2011	**Ślusarczyk M.,** Rybicka B. Role of temperature in diapause response to fish kairomones in crustacean *Daphnia*. Journal of Insect Physiology. IF_{2010} = 2.3
2009	**Ślusarczyk M**. Extended lifespan traded for diapause in *Daphnia*. Freshwater Biology 54: 2252-2262. IF_{2010} = 3.1
2008	**Ślusarczyk M.,** Pietrzak B. To sink or float: the fate of dormant offspring is determined by maternal behaviour in *Daphnia.* Freshwater Biology 53: 569–576. IF_{2010} = 3.1
2006	Pietrzak B., **Ślusarczyk M**. The fate of ephippia - *Daphnia* dispersal in time and space. Polish Journal of Ecology 54: 709-714. IF_{2010} = 0.5
2005	**Ślusarczyk M.,** Dawidowicz P., Rygielska E. Hide, rest or die: a light-mediated diapause response to the threat of fish predation in *Daphnia magna*. Freshwater Biology 50: 141-146. IF_{2010} = 3.1
2004	**Ślusarczyk M**. Environmental plasticity of fish avoidance diapause response in *Daphnia magna.* Journal of Limnology 63: 70-74. IF_{2010} = 1.1
2004	**Ślusarczyk M.**, Rygielska E. Fish faeces as the primary source of chemical cues inducing fish avoidance diapause in *Daphnia magna*. Hydrobiologia 526: 231-234. IF_{2010} = 2.0

Other articles publisher after PhD dissertation:

2010 **Ślusarczyk M.,** Pinel-Alloul B. Depth selection and life history strategies as mutually exclusive responses to risk of fish predation in *Daphnia*. Hydrobiologia 643:33–41. IF_{2010} = 2.0

My participation in the publication of the article I estimate at 70%. It consisted of planning the research, collection and analysis of the collected material and writing the manuscript.

2007 Gélinas M., Pinel-Alloul B., **Ślusarczyk M.** Formation of morphological defences in response to YOY perch and invertebrate predation in two *Daphnia* species coexisting in a mesotrophic lake. Hydrobiologia 594:175–185. IF_{2010} = 2.0

My participation in the publication of the article I estimate at 20%. It consisted of planning the research, data collection and analysis and revision of the manuscript.

2007 Gélinas M., Pinel-Alloul B., **Ślusarczyk M.** Alternative antipredator responses of two coexisting *Daphnia* species to negative size selection by YOY perch. J. Plankton Res. 29: 775-789. IF_{2010} = 1.7

My participation in the publication of the article I estimate at 20%. It consisted of planning the research, data collection and analysis and revision of the manuscript.

2006 **Ślusarczyk M.** Pinel-Alloul, Gélinas M. On ultimate reasons for summer diapause of *Daphnia* in a permanent lake. Verh. Internat. Verein. Limnol. 29: 1440-1442.

Not evaluated by JCR.

My participation in the publication of the article I estimate at 60%. It consisted of planning the research, data collection and analysis and writing the manuscript.

2001 Gliwicz Z.M., Ślusarczyk A. **Ślusarczyk M.** - Life-history synchronization in a long-lifespan single-cohort *Daphnia* population of an alpine lake free of fish – Oecologia 128: 368-378. IF_{2009} = 3.5

My participation in the publication of the article I estimate at 15%. It consisted of planning the research, data collection and analysis and revision of the manuscript.

1999 **Ślusarczyk M.** Diapauza jako obrona przed drapieżnictwem (Diapause as a predatory avoidance adaptation). Kosmos 48: 465-476. Not evaluated by JCR

Articles included in PhD dissertations:

2001 **Ślusarczyk M**. Food threshold for diapause in *Daphnia* under the threat of fish predation. Ecology 82: 1089-1096. IF_{2010} = 5.1

1999 **Ślusarczyk M.** Predator induced diapause in *Daphnia magna* may require two chemical cues. Oecologia 119: 159–165. IF_{2010} = 3.5

1998 **Ślusarczyk, M.** Diapauza jako strategia przetrwania (Diapause as a strategy for survival). Wiadomości Ekologiczne 44: 279-303. Not evaluated by JCR

1995 **Ślusarczyk, M.** Predation-induced diapause in *Daphnia.* Ecology, 76, 1008-1013. $IF_{2010} = 5.1$

Other publications before PhD dissertation:

1997 **Ślusarczyk M.** Impact of fish predation on a small-bodied cladoceran: limitation or stimulation? Hydrobiologia 342/343: 215-221. $IF_{2010} = 2.0$

Total citations of my publications according to the Web of Science on 12.11.2011 = 219

Hirsch Index of my publications according to the Web of Science on 12.11.2011 = 7

DODATKOWE INFORMACJE O WNIOSKODAWCY

A) DANE PERSONALNE

Imię i nazwisko: Mirosław Ślusarczyk
Adres zamieszkania: Panieńska 3 m 6, 03-704 Warszawa
Telefon: 506412358
Adres poczty elektronicznej: m.slusarczyk@uw.edu.pl

B) DZIAŁALNOŚĆ DYDAKTYCZNA

Współprowadzący zajęcia na Wydziale Biologii UW:

- *Biocenozy jeziorne i rzeczne* dla studentów Wydziału Biologii oraz MSOŚ na UW (ćwiczenia i wykłady, współautorstwo programu): 2003-2006, 2008 -2011
- *Ekologia* dla studentów Wydziału Biologii (ćwiczenia): 1992-1995
- *Ekologia* dla studentów międzywydziałowego kierunku bioinformatyka na UW (ćwiczenia i wykłady, współautorstwo programu): 2009-2011
- *Ekologia z Ochroną Środowiska* dla studentów Wydziału Biologii, MSOŚ i MiSMaP (ćwiczenia, współautorstwo programu): 2003-2011
- *Ekologia terenowa* dla studentów Wydziału Biologii, MSOŚ na UW (ćwiczenia, współautorstwo programu): 1992-1995, 2009-2011
- *Hydrobiologia* dla studentów Wydziału Biologii UW (ćwiczenia): 1992-1995, 2002-2011
- *Diapauza* jako strategia przetrwania (wykład monograficzny): 2005, 2007
- *Pracownia magisterska* dla studentów Wydziału Biologii, MSOŚ i MiSMaP (seminarium): 2006, 2008
- *Metodyka nauczania Ekologii* dla nauczycieli akademickich z uczelni rosyjskich w ramach TEMPUS (wykład, ćwiczenia, współautorstwo programu): 2011

Inna działalność dydaktyczna:

- Opiekun obronionych 5 prac licencjackich, 4 magisterskich, 1 doktorskiej (opiekun pomocniczy - Malorie Gelinais na Uniwersytecie Montrealskim: 2002r.)
- Opiekun aktualnie prowadzonych: 1 pracy licencjackiej, 3 prac magisterskich, 1 doktorskiej (opiekun pomocniczy doktoranta – Jacka Radzikowskiego na UW, 2010-2011)

Dotychczas nie zostałem oficjalnie wybrany promotorem pomocniczym żadnego doktoranta

- Recenzent 9 prac licencjackich oraz 6 prac magisterskich
- Prowadzący zajęcia dla wybitnie uzdolnionych uczniów szkół średnich, organizowanych przez Krajowy Fundusz na rzecz Dzieci, 2006 r.

C) WSPÓŁPRACA Z INSTYTUCJAMI, ORGANIZACJAMI I TOWARZYSTWAMI NAUKOWYMI W KRAJU I ZA GRANICĄ

Kontakty naukowe:

- Prof. Korinek, dr M. Cerny, dr A. Pertusek, Uniwersytet Karola w Pradze: 2011
- dr A. Otarov, Institute of Soil Science and Agrochemistry, Almaty, Kazachstan: 2011
- Prof. L. DeMeester, Katolicki Uniwersytet w Luven, Belgia: 2008
- dr K. Więski, Uniwersytet w Huston, USA: 2006-2011
- dr K. Więski, Akademia Rolnicza w Szczecinie: 2004
- Prof. F. Dufresne, Uniwersytet Rimouski, Kanada: 2002
- Prof. Pinel-Alloul, Uniwersytet Montrealski, Kanada: 2001-2011,
- Prof. W. Lampert, Instytut Maxa Plancka, w Ploen, Niemcy: 1997 r.
- dr S. Young, Centrum Biologii Populacyjnej, Uniwersytet Londyński w Silwood Park, 1994 r.
- Prof. T. Lindem, Wydział Fizyki, Uniwersytet w Oslo: 1991 r.

Stypendia naukowe:

2009	Roczne stypendium habilitacyjne Rektora Uniwersytetu Warszawskiego
2008	Dwumiesięczny staż naukowy na KUL (Katolickim Uniwersytecie w Leuven), Belgia, finansowany przez KUL
2005	Roczne stypendium naukowe Rektora Uniwersytetu Warszawskiego za działalność naukową
2004	Stypendium konferencyjne Fundacji Nauki Polskiej
2001-2002	Dwuletnie stypendium typu postdoc finansowane przez GRIL (Groupe de Recherche Interuniversitaire en Limnologie) na Uniwersytecie Montrealskim, Kanada
1995-1999	Stypendium doktorskie finansowane przez KBN na Uniwersytecie Warszawskim
1997	Czteromiesięczne stypendium naukowe finansowane przez DAAD (Deutsche

Akademische Austausch Dienst) w Instytucie Limnologii Maxa Plancka, w Ploen, Niemcy

1994 Półroczne stypendium naukowo-dydaktyczne finansowane przez TEMPUS (Trans-European Mobility Scheme for University Studies) w Centrum Biologii Populacyjnej Imperial College, Uniwersytetu Londyńskiego w Silwood Park, Anglia

Udział w międzynarodowych projektach badawczych:

2010-2011 Wykonawca w projekcie finansowanym z funduszu programu Life UE "Environmental quality and pressures assessment across Europe: The LTER network as an integrated and shared system for ecosystem monitoring"

2002 Kierownik projektu finansowanego przez GRIL (Groupe de Recherche Interuniversitaire en Limnologie)
"Ultimate causes of summer diapause of *Daphnia pulicaria* in the lake Brome",

2001 Kierownk dwóch projektów finansowanych przez GRIL
"Plasticity of spatio-temporal fish avoidance mechanisms in *Daphnia",*"Diapause duration and environmental stability"

1995-1996 Wykonawca pomocniczy w projekcie EU "Controlling the behavior of planktivorous fish with counterfeit information on risk to predation"

D) DZIAŁALNOŚĆ POPULARYZUJĄCA NAUKĘ

Doniesienia na krajowych i międzynarodowych konferencjach naukowych:

2011 Markowska M, Pietrzak B, Bednarska A, **Ślusarczyk M.** Physiological state of *Daphnia pulex* species living in a fish-free high mountain lake measured by RNA:DNA ratio. Plakat**,** międzynarodowa konferencja dot. *Cladocera*, Verbania, Włochy

2011 Bednarska A, **Ślusarczyk M.** Effect of cyanobacteria on eggs abortion in *Daphnia pulicaria* at various thermal conditions. Współudział w ustnej prezentacji**,** międzynarodowa konferencja dot. *Cladocera*, Verbania, Włochy

2011 Pietrzak B, Bednarska A, Markowska M, **Ślusarczyk M.** Physiology, behaviour and extreme longevity differences of *Daphnia* in a fish-free high mountain lake.

Współudział w ustnej prezentacji**,** międzynarodowa konferencja dot. *Cladocera*, Verbania, Włochy

2011 **Ślusarczyk M,** Ochocka A, Cichocka D. Prevalence of diapause response to fish predation cues within *Daphnia***.** Ustna prezentacja, międzynarodowa konferencja dot. *Cladocera*, Verbania, Włochy

2010 **Ślusarczyk M,** Rybicka B. Effect of temperature on diapause response to risk of fish predation in *Daphnia magna.* Plakat, międzynarodowa konferencja Invertebrate Reproduction and Development, Praga, Czechy

2009 **Ślusarczyk M,** Ochocka A. Who cares about overcrowding? Ustna prezentacja, międzynarodowa konferencja *Ressurection ecology*, Szwajcaria

2009 **Ślusarczyk M,** Bernatowicz P. How long to rest in unpredictably changing habitats? Plakat, międzynarodowa konferencja *Ressurection ecology*, Szwajcaria

2007 **Ślusarczyk M**, Pietrzak B, Grabowski T. 2007. Ultimate reasons for deposition of diapausing eggs at the water surface by planktonic *Daphnia*. Ustna prezentacja, kongres SIL (International Association of Limnology), Montreal, Kanada

2007 Bednarska A, **Ślusarczyk M**. 2007. Adaptive timing of diapause in *Daphnia*. Udział w ustnej prezentacji, kongres SIL, Montreal, Kanada

2007 Pietrzak B, **Ślusarczyk M**. 2007. Circadian rhythm of ephippia deposition at the water surface in *Daphnia*. Udział w ustnej prezentacji, kongres SIL, Montreal, Kanada

2006 Więski K, **Ślusarczyk M**. Role of alarm substance and predatory kairomone in predator-induced diapause of the freshwater crustacean *D. magna.* Plakat, kongres Ecological Society of America, Memphis, USA

2005 **Ślusarczyk M.** To sink or float: proximate and ultimate reasons of ephippia appearance at the water surface. Ustna prezentacja, międzynarodowa konferencja dot. *Cladocera*, Szwajcaria

2005 **Ślusarczyk M.** Kairomone induced diapause in *Daphnia*: limited or widespread adaptation? Ustna presentacja, kongres SEFS, Kraków, Polska

2004 **Ślusarczyk M.** Ultimate reasons for summer diapause of *Daphnia pulicaria* in the Lake Brome, Quebec, Kanada. Ustna prezentacja, kongres SIL, Lahti, Finlandia

2003 **Ślusarczyk M.** Rest, hide or fight: conditional response of *Daphnia magna* to a threat of fish predation. Ustna prezentacja, warsztaty “Diapause in aquatic invertebrates”, Verbania, Włochy

2002 **Ślusarczyk M.** Light mediated diapause response of *Daphnia* to danger of fish predation. Ustna prezentacja, międzynarodowa konferencja dot. *Cladocera*, Wierzba, Polska

2002 **Ślusarczyk M.** Diel vertical migration and life history adaptations as mutually exclusive defenses to fish predation in *Daphnia catawba*. Ustna prezentacja, kongres GRIL, Kanada

2000 **Ślusarczyk M.** Extended lifespan and diapause as two contrasting life histories of *Daphnia* in fish-free seasonally changing environment. Ustna prezentacja, warsztaty Biologii Ewolucyjnej, Warszawa, Polska

1997 **Ślusarczyk M.** Predator-induced diapause in *Daphnia* requires two chemical cues. Ustna prezentacja, międzynarodowa konferencja *Diapause in the Crustacea*, Ghent, Belgia

1996 **Ślusarczyk M.** Diapauza jako mechanizm obrony przed drapieżnictwem. Ustna prezentacja, warsztaty Biologii Ewolucyjnej, Warszawa, Polska

1995 **Ślusarczyk M.** The effects of fish pressure on density and demography of a small bodied cladoceran *Bosmina longirostris*. Plakat, międzynarodowa konferencja Shallow Lakes '95, Trophic Cascades in Shallow Freshwater and Brackish Lakes, Mikołajki, Polska

1993 **Ślusarczyk M.** Induction of diapause in *Daphnia magna* by fish chemical stimuli. Ustna prezentacja, międzynarodowa konferencja dot. *Cladocera*, Bergen, Norwegia

Wykłady okolicznościowe w zagranicznych placówkach naukowych:

- Uniwersytet Montrealski, Montreal, Kanada: 2002 r.
- Uniwersytet Cornell, Ithaca, USA: 2002 r.
- Centrum Biologii Populacyjnej Uniwersytetu Londyńskiego w Silwood Park, Wielka Brytania: 1994 r.

Udział w pracach komitetów organizacyjnych konferencji naukowych:

- Krajowego Zjazdu Hydrobiologów Polskich w Warszawie 2003 r.
- Międzynarodowej konferencji Cladocera w Wierzbie, Polska, 2002 r.

Działalność naukowa poza uczelnią:

- Członek Polskiego Towarzystwa Hydrobiologicznego,
- Recenzent czasopism naukowych:

Aquatic Ecology, Biologia, Hydrobiologia, Journal of Limnology, Journal of Plankton Research, Freshwater Biology, Limnology and Oceanography, Naturwissenschaften, PLOS ONE.

E) DZIAŁALNOŚĆ ORGANIZACYJNA

- Członek komisji rewizyjnej Warszawskiego oddziału Polskiego Towarzystwa Hydrobiologicznego: 2011
- Kierownik Stacji terenowej Wydziału Biologii UW w Pilchach, Polska: 2009-2011 (www.hydro.biol.uw.edu.pl/pilchy)
- Członek komitetu Sterującego Budowy Centrum Nauk Biologiczno-Chemicznych Uniwersytetu Warszawskiego: 2009-2011 (www.cent3.uw.edu.pl)
- Członek komisji finansowej na Wydziale Biologii UW: 2009-2011
- Członek komisji ds. informatycznych na Wydziale Biologii UW: 2006-2011

Nagrody za działalność naukową i organizacyjną:

2010	Nagroda Rektora Uniwersytetu Warszawskiego za działalność organizacyjną
2009	Nagroda Rektora Uniwersytetu Warszawskiego za działalność naukową
2008	Nagroda Rektora Uniwersytetu Warszawskiego za działalność organizacyjną
1995	Nagroda Rektora Uniwersytetu Warszawskiego za działalność naukową
1991	Nagroda Polskiego Towarzystwa Hydrobiologicznego za pracę magisterską

Warszawa, 15.12.2011r.

Cykl publikacji „*Diapauza jako mechanizm dyspersji w czasie i przestrzeni u skorupiaków planktonowych z rodzaju Daphnia*" wraz z oświadczeniami autorów o wkładzie pracy w powstanie wspólnych artykułów

2012 **Ślusarczyk M**, Ochocka A, Cichocka D. The prevalence of diapause response to risk of size selective predation in small and large bodied prey species. Aquatic Ecology 00: 000-000. DOI: 10.1007/s10452-011-9376-6
Procentowy udział habilitanta w powstaniu publikacji wyniósł ok. 60%, a polegał w głównej mierze na zaplanowaniu doświadczeń, analizie wyników przeprowadzonych eksperymentów, napisaniu pierwotnej wersji manuskryptu oraz złożeniu manuskryptu do druku.

2011 **Ślusarczyk M,** Rybicka B. Role of temperature in diapause response to fish kairomones in crustacean *Daphnia*. Journal of Insect Physiology.
Procentowy udział habilitanta w powstaniu publikacji wyniósł ok. 70%, a polegał w głównej mierze na zaplanowaniu doświadczenia, analizie jego wyników, napisaniu pierwotnej wersji manuskryptu oraz złożeniu manuskryptu do druku.

2009 **Ślusarczyk M**. Extended lifespan traded for diapause in *Daphnia*. Freshwater Biology 54: 2252-2262.
Procentowy udział habilitanta w powstaniu publikacji wyniósł 100%.

2008 **Ślusarczyk M,** Pietrzak B. To sink or float: the fate of dormant offspring is determined by maternal behaviour in *Daphnia.* Freshwater Biology 53: 569–576.
Procentowy udział habilitanta w powstaniu publikacji wyniósł ok. 60%, a polegał na współudziale w planowaniu i przeprowadzeniu badań terenowych i laboratoryjnych, analizie wyników, napisaniu pierwotnej wersji manuskryptu oraz złożeniu manuskryptu do druku.

2006 Pietrzak B, **Ślusarczyk M**. The fate of ephippia - *Daphnia* dispersal in time and space. Polish Journal of Ecology 54: 709-714.
Procentowy udział habilitanta w powstaniu publikacji wyniósł ok. 40%, a polegał na współudziale w zaplanowaniu i napisaniu artykułu koncepcyjno-przeglądowego,

opartego w znacznej mierze na wynikach wspólnych badań.

2005 **Ślusarczyk M,** Dawidowicz P, Rygielska E. Hide, rest or die: a light-mediated diapause response to the threat of fish predation in *Daphnia magna*. Freshwater Biology 50: 141-146.
Procentowy udział habilitanta w powstaniu publikacji wyniósł ok. 60%, a polegał w głównej mierze na zaplanowaniu eksperymentów, współudziale w ich przeprowadzeniu, analizie wyników, napisaniu pierwotnej wersji manuskryptu oraz złożeniu manuskryptu do druku.

2004 **Ślusarczyk M**, Rygielska E. Fish faeces as the primary source of chemical cues inducing fish avoidance diapause in *Daphnia magna*. Hydrobiologia 526: 231-234.
Procentowy udział habilitanta w powstaniu publikacji wyniósł ok. 60%, a polegał w głównej mierze na zaplanowaniu doświadczeń, współudziale w przeprowadzeniu eksperymentów i analizie wyników, napisaniu pierwotnej wersji manuskryptu oraz złożeniu manuskryptu do druku.

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The prevalence of diapause response to risk of size-selective predation in small- and large-bodied prey species

M. Slusarczyk · A. Ochocka · D. Cichocka

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Abstract The prevalence of diapause response to the simulated threat of fish predation was compared in three species of planktonic crustaceans of the genus *Daphnia* (*D. magna, D. pulicaria* and *D. longispina*), which due to their different body size vary in vulnerability to fish predation pressure in natural conditions. Higher incidence of diapause response was presumed in the larger-bodied species, which due to their higher conspicuousness and higher energetic content experience the greatest size-selective pressure from visually foraging fish. Small-bodied species were expected to utilize less costly yet less effective active defences, e.g. lower conspicuousness achieved due to slower body growth when facing risk of size-selective predation. Proportions of females forming diapausing structures as well as females body size at the maturation period were compared in experimental beakers containing or not containing fish kairomones (chemical cues of fish predation) in a few clonal lineages of 3 species of *Daphnia* derived from a single lake inhabited by fish. The highest incidence of diapause response to fish kairomones was observed in *D. magna* (when measured both as proportion of sensitive individuals and as proportion of inductive clones), the largest of the tested *Daphnia* species. The lowest proportion of individuals and clones that employed diapause was reported in the smallest tested species, *D. longispina.* In addition, the large-bodied *Daphnia* (*D. magna*) showed a greater reduction in body size in response to fish kairomones than the small-bodied species (*D. longispina*). The results of the present study support the assumption of higher prevalence of diapause response to the threat of selective predation in larger and more vulnerable prey species.

Keywords Inducible defences · Predator avoidance · Diapause · Kairomones · Inheritance

Introduction

A periodic delay in ontogenetic development referred to as diapause is a widely used mechanism to ensure survival during periodic deterioration of environmental conditions. Due to the limited physiological demands and specific adaptations associated with the state of developmental arrest, diapausing forms are able to withstand a much wider range of environmental conditions than active organisms: extreme temperatures, desiccation, inundation, depletion of resources, etc. (Danks 1987).

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Strickler and Twombly (1975) first suggested that predation avoidance could be among the functions performed by diapause. Diapausing forms may be more resistant to predation or remain in refuges that are not accessible to predators or active stages. This phenomenon has subsequently been observed in some freshwater crustaceans (Hairston 1987; Slusarczyk 1995; Pijanowska and Stolpe 1996), algae (Rengefors et al. 1998) and a terrestrial mite (Kroon et al. 2008).

Predator avoidance diapause may be induced either in advance of the seasonal, and thus highly predictable, intensification of predatory pressure, or when the first signs of unpredicted intensification of predatory pressure appear in the environment. In the first case, the diapause response may be triggered by token environmental cues indirectly related to the selective force they precede, e.g. by a particular combination of photoperiod and temperature (Hairston and Kearns 1995). In the second, the diapause may be induced by chemical cues associated with predacious activity (Slusarczyk 1995; Kroon et al. 2008).

Planktonic animals apart from diapause may employ a wide variety of behavioural (e.g. dial vertical migration), morphological (e.g. formation of protective spines) and life history (e.g. maturation at smaller sizes) responses to enhance their chances of survival and successful reproduction under predator pressure (Lass and Spaak 2003; Van Donk 2007). The choice of the anti-predation defence response may depend on its costs on one hand and its effectiveness on the other (Harvell 1990). While diapause seems to be one of the most effective protective mechanisms, it imposes extensive costs on the potential prey, e.g. abandoning of development and reproduction, and inability for behavioural reactions. Reckless suspension of development and reproduction may expose the organism to competitive exclusion by other prey that are able to cope with predators in their active form (Hairston and Munns 1984). In a previous study, it was demonstrated that the planktonic freshwater crustacean *Daphnia magna* is more likely to form diapausing eggs in the presence of high concentrations of fish kairomones and when this substance is derived from fish fed conspecific prey (Slusarczyk 1999). The reaction to fish kairomones was also found to be more strongly expressed under unfavourable conditions, such as limited food, low temperature, or in environments lacking a dark refuge (Slusarczyk 2004). For this reason, diapause seems to be utilized as a last resort when active defence appears ineffective or cannot be used, i.e. when the chances of survival and successful reproduction in the active form drop below a threshold where the low benefits assured by diapause make it the best option.

In the present study, we compared the prevalence of diapause response to a simulated threat of fish predation in three closely related freshwater planktonic crustaceans of the genus *Daphnia* (*D. magna*, *D. pulicaria* and *D. longispina)*. The studied species originated from the same lake, where due to their different body sizes they are differentially targeted by fish. Visually foraging planktivorous fish preferentially feed on large-bodied planktonic crustaceans, as they are more conspicuous and provide a greater energetic gain (Gliwicz 2003). Due to its high visibility, the largest of the studied *Daphnia* species (*D. magna*) is hardly ever present in waterbodies inhabited by fish, while the smallest (*D. longispina*) commonly co-occurs with fish in lowland lakes in the temperate zone. It may be hypothesized that larger and thus more jeopardized prey species should exhibit a higher prevalence of diapause response to fish predation risk than smaller species that are able to cope with the predator in their active stage. Small-bodied species were expected to rely more heavily on active defence responses (behavioural, morphological or life history).

Materials and methods

Experimental animals

The experimental clones of three *Daphnia* species (large-bodied *D. magna*, medium-bodied *D. pulicaria* and small-bodied *D. longispina*) originated from diapausing eggs isolated from sediments of the shallow coastal lake Grosser Binnen in North Germany (54°33′09″N, 10°62′38″E). The environmental conditions faced by *Daphnia* in this lake have been described by Lampert (1991). Lake sediments with ephippia (protective carapace structures containing diapausing eggs) were collected and stored in a dark refrigerator for several months prior to the experiment. Ephippia were isolated by sieving the sediments and opened, and diapausing eggs were removed from the protective shells a few weeks before the experiment. It seems that most species of *Daphnia* form ephippial eggs sexually (Zaffagnini

1987). To our knowledge, the formation of ephippial eggs parthenogenetically has not been demonstrated in either *D. magna* or *D. longispina*. Therefore, for these two species, it may be assumed that the two eggs isolated from a single ephippium were genetically unique, but this was not verified by molecular analyses. The ephippial eggs were then exposed individually to conditions known to break diapause: high intensity of fluorescent light with a summer photoperiod (16D:8L) at a moderate temperature (15°C) (Vandekerkhove et al. 2005). The females that hatched from the diapausing eggs began separate clonal lineages of experimental animals. In total, 12 clones of *D. magna*, 2 clones of *D. pulicaria* and 8 clones of *D. longispina* were hatched from diapausing eggs and cultured for experimentation. Some clones originated from common ephippia (4 pairs of clones for *D. magna*, 1 pair of clones for *D. pulicaria* and 3 pairs of clones for *D. longispina*), while the rest were from different ephippia. The ancestors of the experimental animals were kept in standardized conditions in order to minimize the potential effects of environmental variability on the experimental results (epigenetic maternal effect). All experimental animals came from the second brood of the 3rd generation of ex-ephippial females. Each generation of pre-experimental animals came from the second brood and was cultured under favourable conditions: up to 10 sister females of similar age were kept in 0.45-l glass beakers containing water medium supplemented with a high concentration of algal food (*Scenedesmus obliquus*; >1 mg C l^{-1}), at a temperature of 20°C. The medium was exchanged every second day. Experimental females, randomly chosen from a group of synchronously hatched offspring derived from the cohort of 10 sister females, were placed in experimental beakers within 24 h after birth.

Experimental media

The experimental media were based on pond water that had been conditioned by aeration for at least 48 h prior to use. Such conditioning was found sufficient to remove predator kairomones that originated from the field (Loose et al. 1993). Prior to use, the water was filtered through a 0.1-μm filter and supplemented with green algae (*Scenedesmus obliquus*), as food for *Daphnia*, at a final concentration of 0.6 mg C l^{-1}. The kairomone medium was prepared by diluting an extract of planktivorous fish faeces in this control medium to a final concentration equivalent to the faeces excreted by one fish during 7 h in 10 l of water. The extract of fish faeces was prepared a few months before the start of the experiment using a slight modification of the procedure described by Slusarczyk and Rygielska (2004) and stored frozen. Sixty small (10–15 cm) cyprinid fish (crucian carp—*Carassius carassius)* were fed with 60 g of a frozen mixture of the three tested species of *Daphnia* shortly before the collection of faeces. The fish faeces were collected for seven hours and stored at 4°C. They were then homogenized, filtered (0.45 μm), diluted with water to the desired concentration (faeces of 1 fish in 1 ml of extract), aliquoted into daily doses and frozen (−20°C) for future use. This way of kairomones preparation let us control kairomones quality and quantity along as well as across experiments. Such a faecal extract remains inductive for at least 2 years when kept frozen at −20°C (unpublished data).

Experimental set-up

For the experiments, 10 randomly chosen neonates were placed in 0.4 l of water media (pond water with algae) held in 0.45-l beakers. For each clone, three experimental replicates (water media supplemented with fish kairomones) and three control replicates (water media without kairomones) were set-up. The experimental and control water media were exchanged in the beakers every second day (i.e. days 1, 3, 5…), while algae and kairomones were supplied to the beakers every other day (i.e. days 2, 4, 6…).

All beakers were held in a water bath at 20 ± 0.1°C, lit by a fluorescent lamp, and the photoperiod 16L:8D was applied, simulating summer conditions. The number of ephippial females was recorded every second day when the medium in the beakers was exchanged. At the same time, females bearing ephippia were removed from the beakers without replacement. This procedure permitted reliable calculation of the proportion of ephippial females in each treatment and prevented miscalculation due to possible multiple production of dormant eggs by a single female. When individuals were removed, the volume of medium in the beakers was reduced (40 ml per female) in order to keep the animal densities constant over the course of the whole experiment. Newborn offspring were removed from the beakers when the medium was exchanged. Each experiment ran until all females released their 5th brood of eggs (about 4 weeks). As a measure of diapause response, the cumulative proportion of females producing ephippia was scored for each beaker. We were interested not only in mean proportion of responsive individuals but also in proportion of responsive clones within species. By responsive to fish kairomones, we assumed those clones and species that formed significantly more ephippia in beakers with than without fish kairomones.

Measurement of the size at first reproduction (SFR)

When females deposited their first clutch of eggs into the brood chamber, six randomly chosen gravid females from each beaker were measured under a dissecting microscope from the top of the eye to the base of the tail spine to determine size at first reproduction (SFR), also referred to as the body size at the time of maturation.

Statistical analysis

Responsiveness of the tested clones to fish kairomones was determined with pairwise comparisons upon three replicated beakers containing versus not containing fish kairomones. Reduction of body size at time of maturation (SFR) was verified with Student *t* test, while proportion of females forming ephippia was compared with nonparametric Mann–Whitney test for each clone separately. The reaction was considered significant at arbitrary set the $P < 0.05$ level, without corrections for multiple comparisons.

The effect of fish kairomones and species identity on the proportion of ephippial females was verified in the Generalized Linear Model with defined logit link function and binominal error distribution. Numbers of females that produced and that did not produce ephippia in each beaker were used in the analysis. The effect of fish kairomones and species identity on the maturation size (SFR) of experimental females was tested with two-way ANOVA. Mean body size of randomly 5–6 chosen females in each experimental beaker was the response variable.

Nonparametric Spearman rank correlation was used to test for a relationship between the mean body size of females in the control treatments at the time of maturation (SFR) and the diapause response (difference between the proportions of ephippial females in each beaker with kairomones and the mean proportion of ephippial females of a given clone in the control treatments) across all tested clones of *Daphnia*. A similar comparison was performed between the body size in the control treatments at the time of maturation (SFR) and the reduction in maturation body size (relative proportion of the body size in the kairomone and the control treatments in this ontogenetic stage). Statistical evaluations were performed using the STATISTICA v.9 software package.

Results

The diapause response to fish kairomones was observed in all three tested species of *Daphnia* (Fig. 1). The highest proportion of responsive clones was found in *D. magna*, with 8 out of 12 (67%) tested clones showing significant reaction to fish kairomones. The lowest prevalence was observed in the smallest species (*D. longispina*), with 3 out of 8 (38%) tested clones showing significant reaction. In the medium-sized *D. pulicaria*, one of the two tested clones appeared responsive. The body size of experimental females at the time of maturation (SFR) was typically smaller or equal among those treated with kairomones compared with the controls. The SFR was never significantly larger in animals treated with fish kairomones in the present experiment (Fig. 1).

Size at maturation (SFR) of experimental individuals was affected by the presence of fish kairomones (ANOVA, $F_{1,126} = 15.5$, $P < 0.0005$) and by species identity (ANOVA, $F_{2,126} = 1{,}184$, $P < 0.001$) with significant interaction term between the two (ANOVA, $F_{2,126} = 10.6$, $P < 0.001$). Tukey post hoc test revealed significant (at $P < 0.05$) reduction of body size in response to fish kairomones in *D. magna*, yet not in *D. pulicaria*, nor *D. longispina*.

The proportion of ephippial females was affected by fish kairomones on one hand (GLM, Wald Statistic $W_{1,126} = 91.4$, $P < 0.001$) and species identity on the other (GLM, $W_{2,126} = 29.2$, $P < 0.001$) with significant interaction term between the two (GLM, $W_{2,126} = 57.8.6$, $P < 0.001$). The most pronounced reaction appeared in *D. magna* (52.1%) while the least pronounced in *D. longispina* (15.5%). Moreover, species identity was a significant factor in determining the proportion of ephippial females in fish free

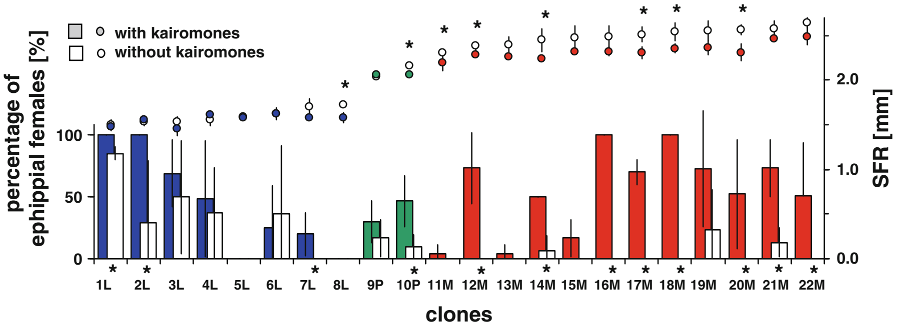

Fig. 1 Proportion of ephippial females (mean ± 1sd) (*bars*) in the experimental clones of three species of *Daphnia* (12 clones of *D. magna*—M, 2 clones of *D. pulicaria*—P and 8 clones of *D. longispina*—L.) when exposed or not exposed to fish kairomones. The clones are arranged in order of increasing mean body size at maturation (SFR; mean ± 1sd) in the control treatment, indicated by *circles* on the graph. *Stars* below the *bars* or above the *circles* indicate significant differences in the measured parameters between the experimental and control treatments for the given clone, determined by pairwise comparison, at $P < 0.05$

conditions (spontaneous diapause) (GLM, $W_{2,113} = 54.5$, $P < 0.001$), with the highest values in *D. longispina* (29.8%) and the lowest in *D. magna* (3.6%) (Fig 2b).

Spearman rank correlation revealed a negative relationship between mean body size at the time of maturation (SFR) in the control treatment and the extent of body size reduction in response to kairomone treatment when tested across all experimental beakers containing fish kairomones ($r = -0.57$, $N = 66$, $P < 0.001$; Fig. 3a). This indicated that the large-bodied species (*D. magna*) reduced their body growth to a greater extent than the small-bodied one (*D. longispina*) when exposed to fish kairomones. SFR was confounded with species identity in this analysis. When tested within separate species, this relationship appeared significant only in case of *D. longispina* ($r = -0.44$, $N = 24$, $P < 0.05$).

Moreover, we found a positive relationship between mean body size at the time of maturation (SFR) in the control treatment and the diapause response to fish kairomones of experimental females in the tested beakers (Spearman rank correlation $r = 0.41$, $N = 66$, $P < 0.001$; Fig. 3b). This confirmed that small-bodied *Daphnia* (*D. longispina*) exhibited a lower diapause response to fish kairomones than large-bodied ones (*D. magna*). Again SFR was confounded with species identity in this analysis. When tested within separate species, this relationship appeared not significant in case of *D. magna nor D. pulex,* while showed negative relationship in *D. longispina* ($r = -48$, $N = 24$, $P < 0.05$). This indicates existence of species-specific effect.

Discussion

Kairomone-induced diapause has so far been reported in a few clones of a single species of *Daphnia* (*D. magna*) originating from the Binnensee. The main aim of this study was to compare the prevalence of this response in three *Daphnia* species (*D. magna, D. pulicaria* and *D. longispina)* of different body size inhabiting this lake. The geographical prevalence of this adaptation is currently being tested in a separate study (unpublished data).

Responsive and nonresponsive clones were identified in all three tested species of *Daphnia*. In line with our expectations, a higher prevalence of diapause response to fish kairomones was found in the large-bodied *Daphnia* than in the small-bodied ones. In the large-bodied species (*D. magna*), the diapause response was observed, on average, in 52% of individuals and in 67% of the tested clones, whereas in the smallest species (*D. longispina*), it was recorded in only 16% of individuals and 38% of clones. In a few highly responsive clones of *D. magna*, 100% of the fish kairomone-treated females formed ephippia. The prevalence of the diapause response to fish kairomones was lower in the clones of the smaller species.

It should be noted that the statistical classification of the responsive clones was based on a low number of replicates (3), which might have underestimated the prevalence of the inductive mechanism among the tested clones and low number of individuals within the replicates (10). However, this should not affect the mean proportion of responsive individuals within

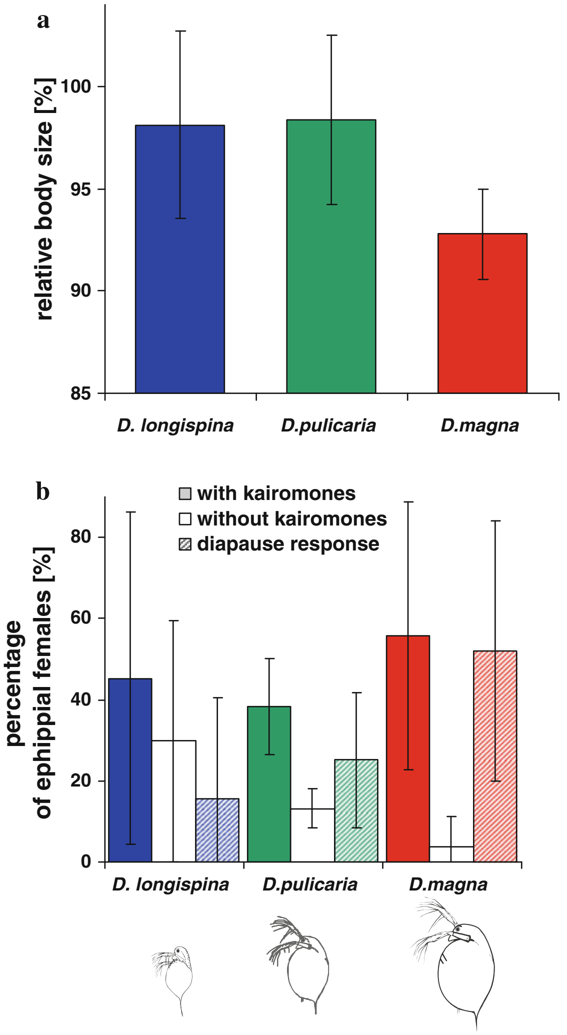

Fig. 2 Life history response of the three tested species of *Daphnia*. **a** Body size response to the presence of fish kairomones (mean ± 1sd) calculated as the proportion of SFR of experimental females in treatment with versus without fish kairomones. **b** Percentage of ephippial females (mean ± 1sd) in the kairomones and the control treatments versus the diapause response calculated as the difference between the earlier two

tested species, which was based on a relatively large sample size (60–360 individuals). Besides, the low number of clones tested (2–12) within the three species only permitted an approximate estimation of the proportion of responsive clones. The considerably greater effort and expense required to produce a more robust estimation of the prevalence of the diapause response to fish kairomones in separate species are beyond the scope of this study. This methodological constraint does not, however, affect our main conclusion concerning the effect of species identity on the incidence of diapause, which was based on a relatively large sample size.

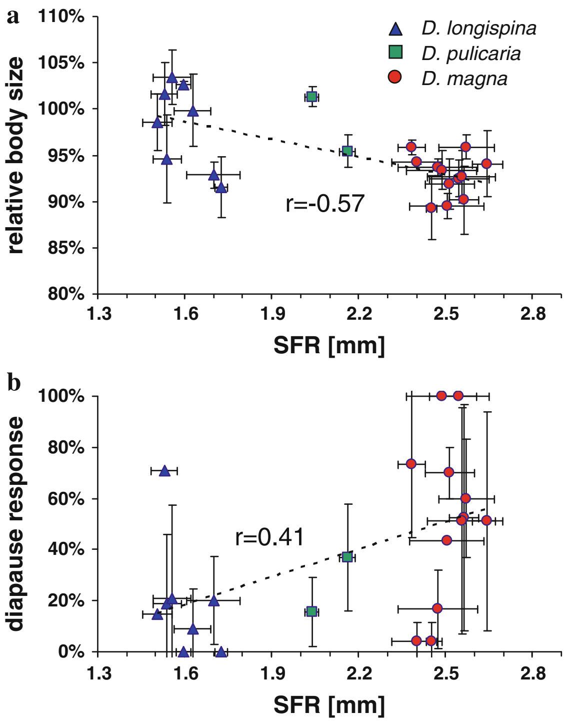

Fig. 3 **a** Relationship between body size at maturation (SFR; mean ± 1sd) in the control treatment versus body size change in response to fish kairomones (mean ± 1sd) for the experimental clones of all tested species of *Daphnia*. **b** Relationship between body size at maturation (SFR; mean ± 1sd) in the control treatment and the diapause response to fish kairomones (mean ± 1sd) for the experimental clones of all tested species of *Daphnia*. *r*—Spearman rank correlation

The low prevalence of the diapause response observed in small-bodied *Daphnia* is in line with the hypothesis presented in the introduction. Large-bodied and thus more conspicuous planktonic organisms may have a greater need for the effective yet costly predator avoidance mechanism of diapause than their smaller and thus less vulnerable relatives, which are able to cope with size-selective predators in their active form. Interestingly, while large-bodied *D. magna* occasionally produced diapausing eggs in the control treatment, this was common in the small *D. longispina*, with some clones producing a high proportion of such eggs. This might indicate either the utilization of a bet-hedging mechanism of diapause control (Evans and Dennehy 2005) or that the control conditions remained less favourable for these animals. However, the further explanation seems unjustified, particularly in light of the fact that under similar conditions the large-bodied *Daphnia*, which require more resources, almost exclusively formed immediately hatching eggs. Of the two strategies that have been considered as alternative ways of diapause control, induction seems to be preferable in predictable habitats, while bet-hedging is thought to be favoured in unpredictably changing environments (Danks 1987). Some of the clones tested in this study seem to combine the two options: they produced a considerable proportion of diapausing eggs in the control treatment and formed even more ephippia in the kairomone treatment (e.g. clones 1L and 2L). The ultimate reason for the formation of diapausing eggs in the control treatment remains unknown, and it may not necessarily be related to predation risk in the native habitat.

In the present study, we observed strong interspecific and intraspecific variation in the diapause response to fish kairomones. This variation may have had different underlying reasons and might have been due to both external (environmental, maternal) and internal (genetic, developmental) factors. In relation to the genetic component of this variation, Lively et al. (2000) suggested that intraspecific variability in inducible reactions to the inductive cue in given conditions may result from genetic polymorphism in either sensitivity or responsiveness to this signal. In the former case, 100% prevalence of the inducible reaction may be observed when organisms are exposed to a strong inductive cue, whereas in the latter case, 100% prevalence may never be reported due to the hypothetical effect of opposing genes that block the inducible reaction in some fraction of the population. Existing polymorphism in responsiveness to the inductive cue might be maintained by frequency-dependent selection or immigration of undefended forms to the exposed population (Lively et al. 2000). The average prevalence of the diapause response barely exceeded 50% of individuals in the most responsive species of *Daphnia* (*D. magna*), despite the exposure of experimental animals to a high concentration of fish kairomones under conditions (low environmental temperature and moderate food) that favoured strong diapause response in former studies (Slusarczyk 2004). This indicates that the variation in the incidence of diapause between experimental clones may be explained by genetic polymorphism in the responsiveness to fish kairomones.

Prior to conducting this study, we presumed that small-bodied prey species exposed to low predation risk might utilize active defences instead of the costly diapause response. However, this scenario was not supported by the experimental results. Contrary to the expected outcome, we observed a more profound reduction in the body size of large-bodied *D. magna* than in the smaller *D. longispina* in response to the fish kairomone treatment. This implies that the two defence strategies examined (slower rate of body growth and formation of diapausing eggs) may be employed more readily by the most vulnerable large-bodied prey relative to small-bodied one.

So far we have considered only the hypothetical effect of genetic polymorphism on the level of variation in diapause response to fish kairomones in the experimental *Daphnia* clones. Environmental fluctuations and the epigenetic maternal effect (Mikulski and Pijanowska 2009) are two other possible reasons for this variation, but these were likely reduced in the present study by standardization of the pre-experimental and experimental protocols. All clones were tested in similar environmental conditions. Moreover, the ancestors of the tested animals were cultured in a similar way for two generations prior to the experiment. These actions should have reduced intraclonal variation (differences between replicates), but they were insufficient to completely eliminate it. The remaining effects of the two factors along with developmental noise were most likely responsible for the observed intraclonal variation in the diapause response to fish kairomones.

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Role of temperature in diapause response to fish kairomones in crustacean *Daphnia*

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A B S T R A C T

The effect of non-lethal thermal conditions on the diapause response to a simulated threat of fish predation was tested in the freshwater planktonic crustacean *Daphnia magna*.

From an early developmental stage, female *Daphnia* were either exposed or not exposed to fish kairomones that notified of the threat of fish predation at the benign growth temperatures of 18, 22 or 26 °C. The proportion of females switching from the production of subitaneous to diapausing eggs and their rate of reproduction were recorded.

Besides the faster development and more intense reproduction observed at higher temperatures, a smaller proportion of the females formed protective, diapausing eggs when exposed to fish kairomones than was the case in colder conditions. The production of diapausing eggs was not observed when the threat of fish predation was absent. These results indicate an interactive effect of fish kairomones and thermal conditions on diapause induction in *D. magna*.

We interpret these findings in the context of strategies for the maximisation of reproductive success. The production of well protected diapausing eggs (which assures low yet stable gains under diverse thermal conditions) may be a more beneficial life history strategy at lower temperatures, where the chances of survival of active individuals until maturation and successful reproduction are low. Higher temperatures permit faster maturation and more intensive reproduction that may surpass numerical losses of active descendants due to predation, making diapause a less rewarding option.

1. Introduction

Diapause can be viewed as a physiological mechanism allowing genome protection during periodic deterioration of environmental conditions that cannot be tolerated by active organisms. The reduced demands and specific physiological adaptations associated with a state of developmental arrest allow dormant forms to tolerate a wider range of hostile abiotic (e.g. extreme temperatures, desiccation) or biotic (e.g. depletion of resources) conditions than active individuals (Danks, 1987). However, diapause seems to be a costly adaptation, as the reckless slowdown of development and reproduction may expose dormant forms to competitive exclusion by lines of organisms that can cope with the selective force in an active form. Thus diapause seems to be used as a final resort, when other less costly mechanisms of active protection cannot be employed or appear ineffective (Slusarczyk, 2004).

Diapause has been proposed as a potential mechanism by which organisms may avoid high predation risk (Strickler and Twombly, 1975). A potential prey that enters diapause may enhance its chances of survival in the face of predatory pressure by becoming unattractive or resistant to consumption, or deposited in locations that are inaccessible to predators and potential prey in the active stage. So far, the phenomenon of predator-avoidance diapause has been demonstrated in a few species of aquatic crustaceans (Hairston, 1987; Slusarczyk, 1995; Pijanowska and Stolpe, 1996) and algae (Rengefors et al., 1998), and in one species of terrestrial mites (Kroon et al., 2008).

The fish avoidance diapause of the freshwater planktonic crustacean *D. magna*, which is the subject of the present study, may be induced by chemical compounds released by hunting fish (Slusarczyk, 1995; Pijanowska and Stolpe, 1996). When exposed to fish-derived chemical cues (fish kairomones), *Daphnia* may switch from the production of active offspring to the formation of diapausing eggs enclosed in a protective shell known as the ephippium. This response is initiated or intensified when *Daphnia* are confronted with kairomones of fish fed conspecific prey, but not necessarily the compounds produced when the fish are fed an alternative diet (Slusarczyk, 1999). The chemical nature of these compounds currently remains unknown.

The diapause response of *Daphnia* to fish kairomones seems to be a flexible reaction, modulated by environmental factors which determine the overall chances of survival and reproduction among active prey individuals: food conditions on the one hand (Slusarczyk, 2001) and the availability of a refuge from predation on the other (Slusarczyk et al., 2005). Here we present further evidence of the modifying effect of thermal conditions: another

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environmental factor of key importance in the decision of a potential prey to produce active vs. dormant offspring when under threat of fish predation.

Temperature may play various roles in the mechanism of diapause induction. "Extreme" temperatures outside the relatively narrow tolerable range for active forms are the most common ultimate reason for diapause induction in living creatures (Danks, 1987). Moreover, cyclically changing temperature (alone or together with photoperiod) may act as a signal heralding the onset of seasonal catastrophic events (Danks, 1987; Gyllström and Hansson, 2004). Lastly, once the environmental temperature determines the kinetics of biochemical processes of ectothermic organisms within the tolerable range of thermal conditions, it may affect the chances of successful reproduction in time-constrained environments, e.g. under predation risk. This final role of temperature was examined in the present study.

When the temperature rises, the rate of reproduction (and other developmental processes) of active organisms increases significantly due to the higher turnover rate and hence shorter generation time (Gillooly et al., 2001). In addition, the outlay on reproduction increases at the raised temperature (Weetman and Atkinson, 2004), if the available resources are not limited. Thermal conditions seem not to affect mortality, nor the rate of development of individuals in diapause (where these processes are controlled by an internal mechanism), or they may only have a minor influence (Danks, 1987; Denlinger, 2002). Therefore, at a given level of predation, the thermal conditions could determine the relative benefits of active life and reproduction compared with diapause, for a potential prey. While dormancy might appear a more profitable strategy at lower temperatures, where the chances of successful reproduction and the survival of active individuals are low, higher temperatures could potentially permit intensive reproduction that surpasses numerical losses of descendants due to predation.

However, thermal conditions might affect not only the reproductive effort of ectothermic prey, but also the energetic demands of its predator, and thus the impact of predation on the prey. Depending on the relative dynamic between the rate of reproduction and the mortality of the prey, temperature might influence the decision of potential prey in different ways. If the reproductive effort of prey individuals increases faster than the risk of mortality due to predation, the water temperature might militate against the decision to enter diapause. In the opposite case, the reverse reaction might be expected.

In the present study we tested the effect of various non-lethal thermal conditions on the diapause response to fish kairomones in the freshwater planktonic crustacean *Daphnia magna*.

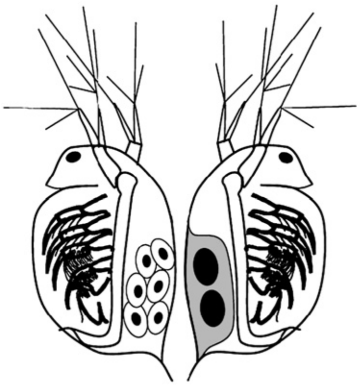

Fig. 1. Sketch of experimental females of *Daphnia magna*. Female with subitaneous eggs on the left, female with ephippium containing diapausing eggs on the right.

2. Methods

2.1. Experimental animals

Experimental *D. magna* used in this study, originated from the shallow, eutrophic Binnensee (Schleswig Holstein, North Germany), where it coexists with fish. *D. magna* is a filter feeding planktonic crustacean inhabiting freshwater habitats (Fig. 1). In favourable periods it produces successive broods of directly developing parthenogenetic eggs. When conditions deteriorate, females may produce diapausing eggs (formed typically by meiosis). The diapausing eggs are enclosed in a protective carapace shell called the ephippium, which is released into the environment by moulting females.

2.2. Experimental setup

The experiment was performed using a flow-through system with closed circulation of medium containing fish kairomones, or control medium lacking chemical fish cues (Fig. 2). The experimental animals were kept in glass chambers of 250 ml capacity through which flowed one of the two media. Experimental chambers were maintained at one of three different thermal conditions by placing them in temperature controlled water baths set at 18, 22 or 26 ± 0.1 °C. The temperature in the three water baths was monitored at 1 min intervals using a multi-channel thermal recorder. Each water bath contained 6 chambers: triplicates with kairomone medium and triplicates with control medium. The

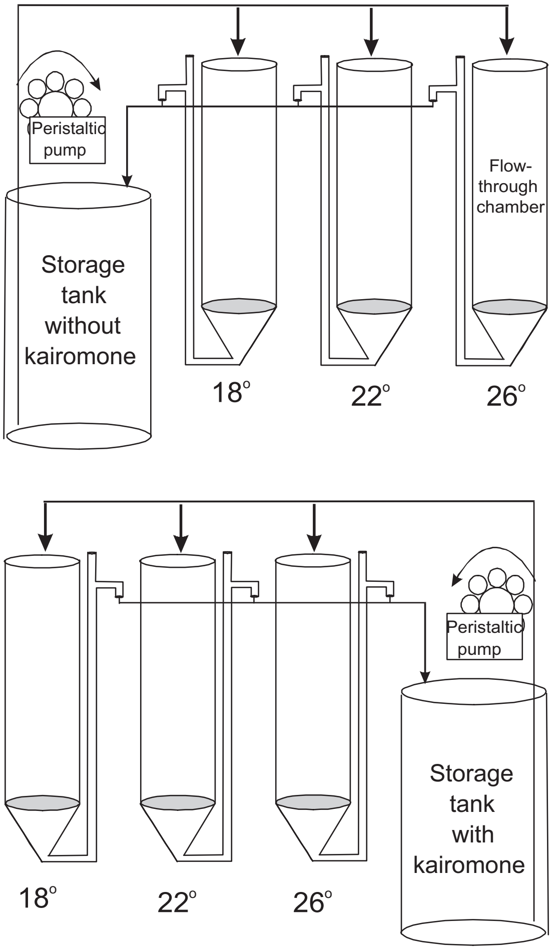

Fig. 2. Schematic diagram of a single replication of the experimental flow-through system.

outflowing medium from the first replicate chamber at a given temperature mixed with the outflow from the first replicate chambers at the two other temperatures in a common storage. In the same way, the outflowing medium from the second replicates from all three temperatures mixed together in a common storage tank, as did the medium from the third replicates. Kairomone media were never mixed with control media lacking fish cues. Thus, three storage tanks for medium with fish kairomones and three storage tanks for control medium were employed. The media were pumped back into the chambers using three multichannel peristaltic pumps at high flow rate (300 ml per experimental chamber per hour), the separate pump for each replication. Closed circulation and the mixing of experimental media from the three temperature treatments was used to reduce potential side effects of the thermal conditions, i.e., faster depletion of food resources due to increased filtration rates by *Daphnia* and more rapid decomposition of fish kairomones at higher temperature. In this way, food concentrations and fish kairomones were maintained at similar levels in the different thermal treatments. The set-up was illuminated with cool fluorescent light (approximate intensity 5.7 μmol m^{-2} s^{-1}) with a summer photoperiod (16L:8D).

2.3. Experimental media

Experimental media were based on a pond water that had been aerated for at least 48 h to remove any predator kairomones (Loose et al., 1993). Prior to use, the water was filtered through a 0.3 μm filter and supplemented with green algae (*Scenedesmus obliquus*) as food for the *Daphnia*, at a final concentration of 0.3 mg C L^{-1} that limited reproduction yet not survival of the experimental animals. Since chemical nature of fish kairomones and alarm substances remain unknown they were collected in natural way using a slightly modified procedure described by Slusarczyk and Rygielska (2004). The kairomone medium was prepared by diluting a defrosted extract of fish faeces in the control medium to a final concentration equivalent to the faeces from 1 fish produced within 8 h per 10 L of water. The frozen extract of planktivorous fish faeces was prepared a few days before the start of the experiment. Seventy small (10–15 cm) cyprinid fish (including rudd – *Scardinius erythrophtalmus* and crucian carp – *Carassius carrasius*) were fed with given number of conspecific *Daphnia* (150 *Daphnia* per fish) shortly before the collection of faeces started and then with chironomid larvae (0.7 g of larvae per fish in total). The fish faeces were collected for eight hours and stored at 4 °C. Then the faeces were homogenised, filtered (0.45 μm), diluted with water to the desired concentration (faeces of 1 fish in 1 ml of extract), aliquoted into daily doses and frozen (−20 °C) for future use. Such an extract of fish faeces remains inductive for at least 2 years after preparation when kept frozen at −20 °C (unpublished data).

2.4. Preparation of experimental animals

The experimental individuals came from a line of sister females reproducing parthenogenetically, derived from a single diapausing egg hatched three months before the experiment started, and so were most likely genetically identical. A single clone of *Daphnia* was used in the experiment to preclude any effect of genetic differences between the experimental animals. Since not all clones of *D. magna* inhabiting Binnensee produce diapausing eggs in response to fish kairomones (unpublished data), the experimental clone was pre-selected for positive diapause reaction to fish cues prior to the experiment.

The mothers of the experimental animals (about 60 sisters of similar age) were kept in common conditions to preclude variation of experimental results due to maternal effects. They were kept in a common 3 L beaker in aqueous medium that was exchanged daily, at an ambient temperature of 22 °C in the presence of a high concentration of algal food (>2 mg C L^{-1} initially).

2.5. The experimental procedure

The experimental females, chosen at random from a group of synchronously hatched offspring were placed in the flow-through chambers (30 neonates in each one) within 20 h of being born.

At the point when the experimental females had matured and deposited their first clutch of eggs into the brood chamber, six gravid females, randomly chosen from each chamber, were measured (from the top of the eye to the base of the tail spine) under a dissecting microscope. This dimension was taken as the size at first reproduction (SFR). The time of deposition of the first clutch of eggs into the brood chambers by the experimental females was taken as the age at first reproduction (AFR). This was recorded at 24 h intervals. Newborn offspring were removed from the experimental chambers without replacement and counted each day. This allowed calculation of the rate of production of active offspring by experimental females, expressed here as the number of active offspring produced by a single female per day. Ephippial females were removed from the experimental chambers (without replacement) to reliably calculate the percentage of these females, and avoid miscalculations due to the possible multiple production of diapausing eggs by a single female. In order to maintain experimental females in a constant volume throughout the experiment, the total volume of circulating media was reduced by 45 ml for every animal removed from a chamber. Clearly, the numbers of females in the experimental chambers varied with time. However, the high flow-rate of the experimental media should have prevented significant differences in the concentration of resources between chambers due to the unequal numbers of individuals. The experiment was continued for four weeks. The experimental media were changed every day by replacement with fresh media at the rate of 45 ml per experimental female.

2.6. Statistical analysis

Statistical analysis was performed with the factorial ANOVA with use of the presence/absence of fish kairomones and environmental temperature as categorical predictors. Data on the proportions of ephippial females was arcsine square-root transformed prior to analysis.

3. Results

Growth temperature affected all of the measured life history parameters of the experimental *Daphnia*, while fish kairomones affected most of them. Growth temperature influenced maturation size (SFR) of the experimental females (ANOVA: $F_{1,12} = 40$ $P < 0.00005$, Fig. 3). The SFR of *Daphnia* was reduced at the successively higher temperatures in media with and without fish kairomones. A trend towards smaller body size of the experimental females was observed in the fish treatment, but this was not significant. Growth temperature had a significant effect on the maturation age of experimental females (ANOVA: $F_{1,12} = 257$, $P < 0.0001$, Fig. 3). As the temperature increased, the maturation age of the females decreased. A trend towards faster maturation of experimental females in the fish kairomone medium was also observed, but this was not significant again. Growth temperature had a significant effect on reproductive output in the initial brood (ANOVA: $F_{1,12} = 6.8$, $P < 0.05$, Fig. 3). The number of eggs in the initial brood was the highest at the lowest temperature and decreased with rising temperature. The females in the fish kairomone medium produced more offspring at all tested temperatures; yet the effect of fish chemical cues on the clutch

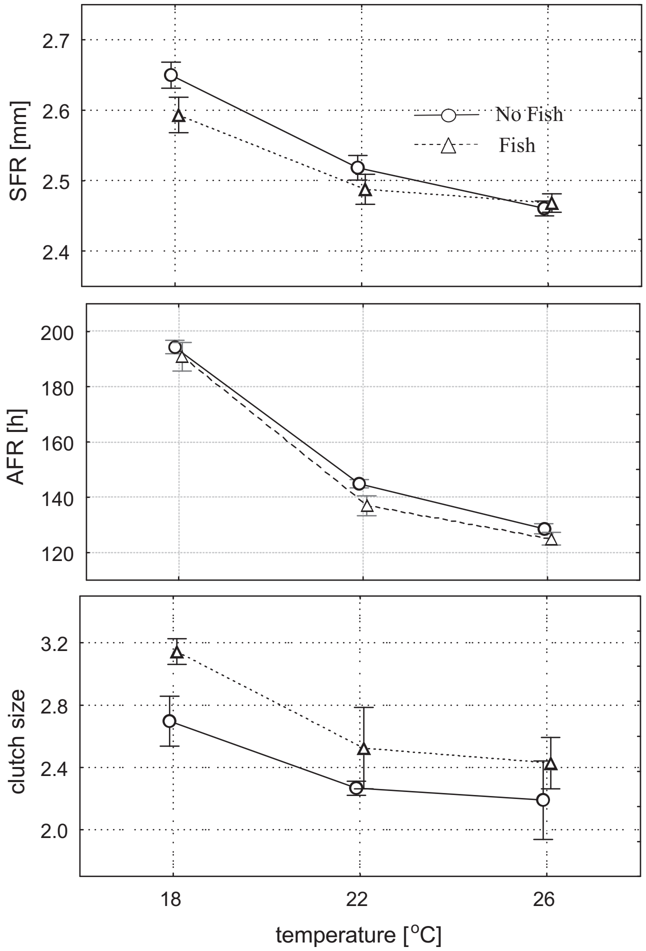

Fig. 3. Size (top panel), age (middle panel) and clutch size (bottom panel) (mean ± 1 SE) of primiparous *Daphnia* in media with or without fish kairomones at different growth temperatures.

size of the initial brood was marginally significant (ANOVA: $F_{1,12} = 4.6$, $P = 0.05$). With increased growth temperature, the reproductive rate increased (ANOVA: $F_{1,12} = 6.7$, $P = 0.01$). In the control medium, this trend was not quite clear at the highest tested temperature due to reproductive depression in one of the experimental chambers. The rate of *Daphnia* reproduction in the fish treatment appeared marginally higher compared to the control medium (ANOVA: $F_{1,12} = 4.6$, $P = 0.05$). Interaction terms between temperature and fish kairomones remained insignificant in the listed parameters.

Both growth temperature and fish kairomones had a significant effect on the proportion of females that formed ephippia during the study period. The production of ephippia was observed in neither the control medium nor the fish kairomone medium at 26 °C. With a decrease in growth temperature, the proportion of females forming ephippia increased in the fish kairomone medium, from 0% at 26 °C, 29% at 22 °C, up to 59% at 18 °C (Fig. 4). Therefore both growth temperature (ANOVA: $F_{1,12} = 25.7$, $P < 0.0005$) and fish kairomones (ANOVA: $F_{1,12} = 90.5$, $P < 0.0005$) had a significant effect on the proportion of ephippial females with significant effect of the interaction term (ANOVA: $F_{1,12} = 25.7$, $P < 0.0005$).

4. Discussion

Unsurprisingly, the growth temperature and the presence of fish kairomones had a strong effect on most of the measured life history parameters of the experimental *Daphnia*, i.e. age and size at maturity, and reproductive rate. Similar reactions have been observed in previous studies on *Daphnia* responses to fish kairomones (e.g. Sakwinska, 1998; Lass and Spaak, 2003). Faster maturation at smaller sizes and higher reproductive rate at higher temperature are likely physiological consequences of a faster metabolic rate. Faster maturation at smaller sizes and higher reproductive rate in the presence of kairomone may be interpreted as results of a life history strategy maximising reproductive success where there is a risk of predation, i.e. the effect of conditional polyphenism *sensu* (Walker, 1986). Lower investment in body size growth and faster maturation under the threat of size selective predation posed on a larger prey might reduce predatory pressure and increase the prey chances of survival until reproduction. Reduced investment in growth may free resources that can be invested in reproduction and might explain the higher clutches observed in the fish kairomone treatment group.

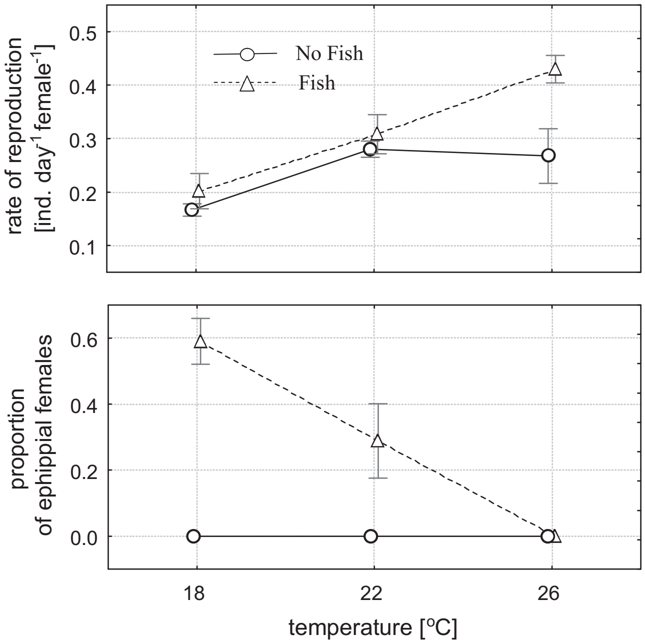

Fig. 4. Daily rate of active offspring production (mean ± 1 SE) by a single female within the first two weeks of the experiment in media with or without fish kairomones at different growth temperatures (top panel); mean frequency of occurrence of experimental females producing ephippia (mean ± 1 SE) under the same experimental conditions during the course of the experiment (bottom panel).

In addition in the present study, growth temperature was shown to influence the frequency with which *Daphnia* produce diapausing forms when exposed to the simulated threat of fish predation. We interpret this novel finding in the context of strategies maximising fitness under the threat of predation in different thermal conditions.

At low risk of fish predation with a high chance of survival of active offspring, the production of diapausing eggs has considerable costs with few benefits, when thermal conditions are within a tolerable range. However, under high predation risk, when the chances of survival of potential prey until reproduction are limited (but higher than zero), the relative gains due to the production of active vs. dormant offspring might depend on environmental conditions that determine the likelihood of successful reproduction of the active prey, e.g. water temperature. At high yet favourable temperatures, the high rate of prey development and reproduction could overcome numerical losses among descendants due to predation and might represent a more beneficial strategy than the formation of a few protective yet inactive ephippial eggs (*Daphnia* may produce at most two diapausing eggs, compared with dozens of subitaneous eggs in a single clutch). In comparison, at lower temperatures and considerable risk of predation, the consequently reduced rate of development and reproduction leading to the production of low numbers of active offspring may decrease the reproductive success of a prey compared with the production of fewer – but well protected – diapausing eggs. This line of reasoning would be valid in the case of temperature-independent prey mortality, or when the mortality of a prey changes less rapidly with temperature than its rate of reproduction. Otherwise, the opposite effect might be expected, i.e. increased production of ephippial eggs by *Daphnia* with rising temperature.

Although the impact of thermal conditions on the outcome of the fish-*Daphnia* interplay was not directly assessed here, the nature of this impact may be predicted from indirect sources. According to Gillooly et al. (2001), temperature affects metabolic rates in invertebrates to a greater extent than in fish. Furthermore, seasonal changes in thermal conditions may affect the population growth rate of the prey and predator differently where there is considerable divergence of their generation times. This should increase any difference between prey reproduction and mortality even further. The rate of reproduction of organisms has a multiplicative nature. A change in temperature within a season may alter the population growth of a multivotine prey of short generation time (e.g. *Daphnia*) exponentially (both functionally and numerically), while that of a univoltine predator (e.g. fish) can only change linearly (functionally). Thus temperature might affect the rate of *Daphnia* reproduction to a greater extent than its mortality due to fish predation.

The influence of thermal conditions on the rate of production (Lass and Spaak, 2003) and decomposition of fish kairomones (Loose et al., 1993) may also affect the *Daphnia* response to these chemical cues. A stronger life-history reaction of *Daphnia* to fish kairomones was observed by Lass and Spaak (2003) when these compounds were produced at a higher temperature. This effect was explained by the more intensive production and increased accumulation of fish exudates in warmer conditions. On the other hand, a less intense behavioural reaction to fish cues was recorded in *Daphnia* when kairomone was kept under non-sterile conditions in a warmer climate prior to use, indicating decomposition of the active compounds by bacteria (Loose et al., 1993). In the present study, the high rate of recirculation of the medium common to all thermal treatments should have maintained the concentration of fish kairomones at a similar level. Under such conditions, the lower frequency of ephippial females observed at high temperature in the fish treatments may be explained entirely by the direct effect of thermal conditions on the reproductive output of *Daphnia*, with the proliferation of active offspring favoured over the formation of diapausing eggs.

Interestingly, the tested gradients of various environmental conditions, i.e. temperature (present study), food concentration (Slusarczyk, 2001) or light intensity (Slusarczyk, 2004), caused a gradual change in the prevalence of the diapause response to fish kairomones and not a sharp transition from 0 to 100% at a certain threshold value. This indicates that an incremental change in environmental conditions gradually affects the chances of survival and reproduction of active prey and that they may be "aware" of this. However, why was 100% prevalence of ephippial females never observed in this experiment? The possibility that by reducing the temperature 100% of experimental females treated with fish chemical cues could be stimulated to form diapausing eggs cannot be excluded. However, our recent study (unpublished data) indicates that separate clones of *D. magna* from the Binensee may vary in the prevalence of the diapause response to fish kairomones from 0 to 100% under stimulating environmental conditions.

What might be the adaptive function of a flexible temperature-dependent diapause response to the risk of fish predation in *Daphnia*? In lakes of the temperate zone, both thermal conditions and the risk of fish predation may vary considerably within and between seasons (Sommer et al., 1986; Hairston, 1988). Moreover, both factors may change dramatically along a vertical gradient in these lakes. A flexible temperature-dependent diapause response to fish kairomones could provide *Daphnia* with an adaptive reaction to a broad spectrum of environmental conditions that may be encountered in spatially and temporally heterogeneous bodies of water.

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Oświadczam, że mój udział w powstaniu artykułu:

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Extended lifespan traded for diapause in *Daphnia*

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SUMMARY

1. Most freshwater crustaceans of the genus *Daphnia* are cyclically parthenogenetic organisms that are well adapted to unstable habitats due to their short life cycle, wide phenotypic plasticity, and the ability to produce protective diapausing eggs in anticipation of environmental deterioration. Short lifespan and heterogonic reproduction are typical features of *Daphnia* in a broad spectrum of freshwater habitats, from small temporary pools to large permanent lakes. However, in some locations, departures may be observed from this typical life history pattern to obligate asexuality or extended lifespan.
2. A 3-year field study in a deep ultraoligotrophic fish-free alpine-type lake (Czarny Staw in the Tatra mountains in southern Poland) revealed the coexistence of two closely related asexual lineages of *Daphnia* of the *pulex* complex, which differ in body colour (transparent versus orange) and in their strategies for surviving long winters.
3. The 'transparent' clone of European origin exhibits an ephemeral lifestyle. It completes its life cycle within a single season, forming two generations of active specimens during the short summer and producing diapausing eggs late in the season. Transparent individuals live no longer than 5–6 months in this cold lake and survive winter exclusively in the form of diapausing eggs.
4. Individuals of the 'orange' lineage, which are closely related to eastern Nearctic *Daphnia pulicaria*, exhibit a biennial lifestyle unique to this genus. They do not form diapausing eggs or produce them only occasionally. Instead, they are active throughout the year and live for up to 13–14 months. Reproduction is postponed until the second year of life when food conditions have improved. Rich lipid reserves accumulated in the first season fuel them during the long winter and permit early reproduction the following spring.
5. Possible reasons for the evolution of obligatory parthenogenesis and long lifespan in *Daphnia* are discussed.

Keywords: cyclical parthenogenesis, diapause, fishless habitats, lifespan evolution, mountain lake

Introduction

Habitat instability seems to be one of the key factors determining the life histories of living creatures. Frequently changing habitats typically host opportunistic species that can complete their life cycles within short periods of favourable conditions, whereas more constant environments favour long-lived forms (Pianka, 1970; Grime, 1977).

Water fleas of the genus *Daphnia* are small filter feeding planktonic crustaceans that are well adapted to changing conditions in freshwater habitats due to their short life cycle, wide phenotypic plasticity and heterogonic reproduction (i.e. cyclical alternation between asexual and sexual reproduction). In favourable periods they proliferate rapidly as a result of their fast maturation, high fecundity and parthenogenetic reproduction. Their sexual phase is typically confined to unfavourable seasons and concludes with the formation of diapausing eggs held in a protective ephippial case. Cyclical parthenogenesis seems to combine the best aspects of the two modes of reproduction, while avoiding their pitfalls. Parthenogenesis

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offers the benefits of fast proliferation, but when obligatory it is prone to the accumulation of deleterious mutations ('Muller's ratchet effect'; Muller, 1932). The sexual phase permits the removal of deleterious mutations and enhances genetic diversity, but it slows reproduction and breaks up favourable gene combinations (Lewis, 1987).

Short lifespan and heterogonic reproduction in Daphnidae appear to be successful in a broad spectrum of freshwater habitats, from small temporary pools to large permanent lakes. In some locations, however, departures from this typical life history pattern may be observed. One is obligatory parthenogenesis (apomixis), while another is extended lifespan. The switch from heterogony to obligatory parthenogenesis (i.e. the production of both subitaneous and diapausing eggs in an asexual manner) seems the more common of these two rare classes. It may be caused by either of the following mechanisms that lead to meiotic disruption in females: (i) introgressive hybridisation commonly combined with poliploidisation (Dufresne, 1994); (ii) contagious asexuality (i.e. female-expressed dysfunctional mutation transferred sexually by male individuals) (Innes & Hebert, 1988; Paland, Colbourne & Lynch, 2005); (iii) spontaneous mutations or (iv) parasite infection (Simon *et al*., 2003). These reasons for the occurrence of obligatory parthenogenesis may indicate its accidental origin and possible unfavourable effects. However, the prevalence of obligatorily asexual forms in marginal habitats (e.g. at high latitudes or altitudes; Edmondson, 1955; Weider *et al*., 1999; Hobaek & Weider, 1999; Aguilera *et al*., 2007) demonstrates its favourable outcome.

A more profound departure from the typical life history pattern has recently been observed in *Daphnia* in permanent cold mountain lakes. Unlike most other water fleas that live for no more than a few weeks in their natural habitats, a biennial lifespan was reported in *Daphnia* living in these remote environments (Gliwicz, Slusarczyk & Slusarczyk, 2001; Larsson & Wathne, 2006). In both habitats *Daphnia* overwinter in active form and postpone reproduction till the second year. The lifespan of living creatures seems to be a flexible trait that is prone to selection. Short lifespan appears advantageous in frequently changing environments, while a long lifespan with late or prolonged reproduction suits safe permanent habitats or locations where environmental conditions remain unsuitable for young individuals (Williams, 1957; Williams *et al*., 2006). What makes *Daphnia* change from a life history pattern that is successful in so many different habitats? The present study examines the co-occurrence of two 'rare' life history patterns (obligate asexuality or long lifespan) in two closely related asexual lineages of *Daphnia* inhabiting a high mountain lake lacking fish. The coincidence of these two unique life history options in a single habitat may help us to reveal the ultimate reasons for their selection.

Long lifespan and permanent asexuality in *Daphnia* inhabiting the ultraoligotrophic high mountain lake Czarny Staw in the Tatra Mountains was described almost a century ago by Minkiewicz (1911). The findings of our recent study (Gliwicz *et al*., 2001) supported this claim by demonstrating the coexistence of two strategies to survive the long winter (as diapausing eggs or long-lived active forms) in what were probably asexual *Daphnia* inhabiting this lake. At the time of this previous investigation it was not known if the two strategies were employed by individuals of a common population or by distinct lineages. Therefore, some intriguing questions remained open: what was the reason for the change from the typical heterogonic reproduction, and why do long-lived forms produce diapausing eggs if they already have an alternative solution to the challenge of the long winter? The aim of the present study was to answer these questions.

Many years ago Minkiewicz (1911) reported the co-occurrence of two *Daphnia* colour morphs in Czarny Staw. Furthermore, a recent study indicated that the population of *Daphnia* in this lake is comprised two obligatorily parthenogenetic clones of the pulex group (Cerny, 1995). Therefore, at the start of the present study it was assumed that the two overwintering strategies might be employed by two reproductively isolated *Daphnia* lineages in this lake. To test this assumption, the fate of both colour morphotypes in Czarny Staw was followed over three consecutive years (1996–98).

Methods

The lake and its zooplankton

Lake Czarny Staw pod Rysami is located in Dolina Rybiego Potoku (Fish River Valley) in the Polish Tatra mountains at an elevation of 1581 m a.s.l., just above the timberline zone (49°18′90″N, 20°07′61″E). It is a fish-free, ultraoligotrophic, alpine-type lake of post-glacial origin, of maximum depth 76 m and area 21 ha. Secchi disc transparency ranges between 10 and 24 m. The lake is covered by a thick (up to 1.5 m) layer of ice and snow from December until June. In the absence of fish, the macro-zooplankton community is very simple, with one invertebrate predator *Cyclops abyssorum* tatricus (Kozminski) and a single genus of filter feeding herbivorous cladocerans (*Daphnia* of the *pulex* species complex), occasionally accompanied by smaller cladocerans: *Holopedium gibberum* Zaddach and *Bosmina longirostris*, Müller and the rotifer *Asplanchna priodonta* Gosse during the summer.

In a previous study, we reported the presence of two colour morphs of *Daphnia* in this lake: orange and transparent (Gliwicz *et al.*, 2001). We called them both *Daphnia pulicaria* Forbes due to morphological features typical of this species. Electrophoretic analysis of protein markers made in the present study and recent DNA analysis (Markova *et al.*, 2007; M. Slusarczyk and J. Mergeay, unpubl. data) indicate that the two colour morphs represent distinct lineages. Markova *et al.* (2007) suggested that the two colour morphs are of different origin: orange individuals are closely related to an eastern Nearctic clade of *D. pulicaria*, while the transparent morphs are more closely related to a European clade of the *tenebrosa* group.

Sampling

Samples were collected from Czarny Staw at monthly intervals from late summer 1996 to autumn 1998. All water samples were collected and thermal-oxygen depth distributions measured via a hole made in the snow-ice cover or from an inflatable boat anchored at the deepest point of the lake. Temperature and dissolved oxygen concentration were measured using a YSI 5740 oxygen probe (YSI Co., Yellow Springs, OH, U.S.A.). Lake water for organic carbon estimation was taken with a 2 L Patalas sampler (Selfmade instrument) from depths of 2.5, 10 and 20 m, and pooled together. To estimate particulate organic carbon (POC) in the <50 μm fraction (considered to be a measure of food available to *Daphnia*), 2 L volumes of the mixed lake water collected in 1997–98 were pre-filtered through 50 μm mesh-size net and then particulate material was collected on glass-fibre GF/F filters (Whatman, Inc., Clifton, NJ, U.S.A.). POC on three replicate filters was measured using a UNDR infrared gas analyser after combustion in a Pregl-Roth oven (Krambeck, Lampert & Brede, 1981). Data for 1996–97 are not available.

Zooplankton samples (three replicates on each date) were collected by vertical hauls from a depth of 45 m to the surface using a quantitative Apstein plankton net (Selfmade instrument) of 0.2 mm mesh size, equipped with a conical annex of 6 dm^2 inflow area, giving a sampled volume of 2700 L. The seasonal study focused on the population of *Daphnia*. Zooplankton samples preserved in 4% sugar–formaldehyde were counted under a dissecting microscope to determine *Daphnia* density. Single non-preserved zooplankton samples were examined to determine population parameters. Since body lipids and orange pigment decay rapidly (usually within 48 h), non-preserved samples were processed within 12 h of collection. First, 100–150 randomly chosen *Daphnia* were inspected for sex, body length (upper edge of eye to base of tail spine), clutch size (number of eggs or embryos per clutch), the presence of ephippia and visible lipid reserves (Tessier & Goulden, 1982). The inspected animals were classified into two morphotypes according to body colour (presence/absence of orange pigment in body lipids; most likely of carotenoid origin; B. Pietrzak, pers. comm.) and also scored according to the amount of visible lipid droplets in the body: 0, 1, 2, 3, 4 or 5, from low to high body lipid (Fig. 1), as in our previous study (Gliwicz *et al.*, 2001). The original scale for the visible body reserves index (0, 1, 2 and 3) proposed by Tessier & Goulden (1982) for American *Daphnia* was expanded due to the higher visible lipid content in orange *Daphnia* from Czarny Staw. The lipid index may indicate *Daphnia*

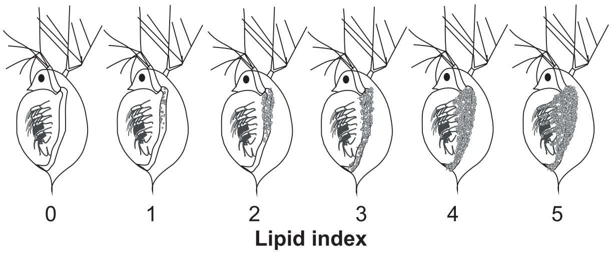

Fig. 1 Visible lipid reserves of *Daphnia* from Czarny Staw scored according to a lipid index, 0–5 (Gliwicz *et al.*, 2001; extended from the scale of Tessier & Goulden, 1982). Lipid droplets, visualised as small round bodies, were counted in 100–150 randomly chosen *Daphnia*.

performance. Tessier & Goulden (1982) found that older specimens in good shape typically had a higher lipid index than younger or malnourished individuals.

Thirty randomly chosen orange and thirty transparent *Daphnia* individuals collected in October 1997 were analysed a few months later for the presence of allozymes of aldehyde oxidase (EC 1.2.3.1) and arginine phosphokinase (EC 2.7.3.3), which according to Cerny (1995) should discriminate clones inhabiting the lake. Electrophoresis was carried out on Titan III cellulose acetate plates (Helena Laboratories, Beaumont, TX, U.S.A.) using a standard method described by Hebert & Beaton (1989).

Results

The water temperature in Czarny Staw changed only moderately throughout the year (Fig. 2). However, POC concentration in seston particles smaller than 50 μm, taken as a measure of available food for *Daphnia*, fluctuated markedly. The highest POC values were recorded in late June/early July, shortly after the ice cover had melted completely, and levels then decreased gradually until they became undetectable in the middle of the following May, just before the next ice cover melt (Fig. 2). Oxygen concentration never dropped below 7 mgL^{-1} in the upper 40 m of the lake.

Table 1 Results of electrophoretic analysis of allozymes of aldehyde oxidase (AO) and arginine phosphokinase (APK) in the two colour morphs of *Daphnia* from Czarny Staw

Daphnia morphotypes	APK	AO	Sample size
Transparent	mf	ff	30
Orange	ff	sf	30

Electrophoretic analysis of allozymes indicated that pigmentation associated with body lipids (high transparency versus orange colour) reliably discriminated different lineages of *Daphnia* in the lake (Table 1). Unfortunately, due to a lack of other evident morphological differences between the two colour morphs, it proved impossible to classify the smallest individuals and specimens with no lipid reserves by visual inspection.

The two *Daphnia* colour morphs differed in their strategies for surviving the long winter. While orange individuals were present in the lake throughout the study period, the transparent specimens were not recorded in the open water between February and

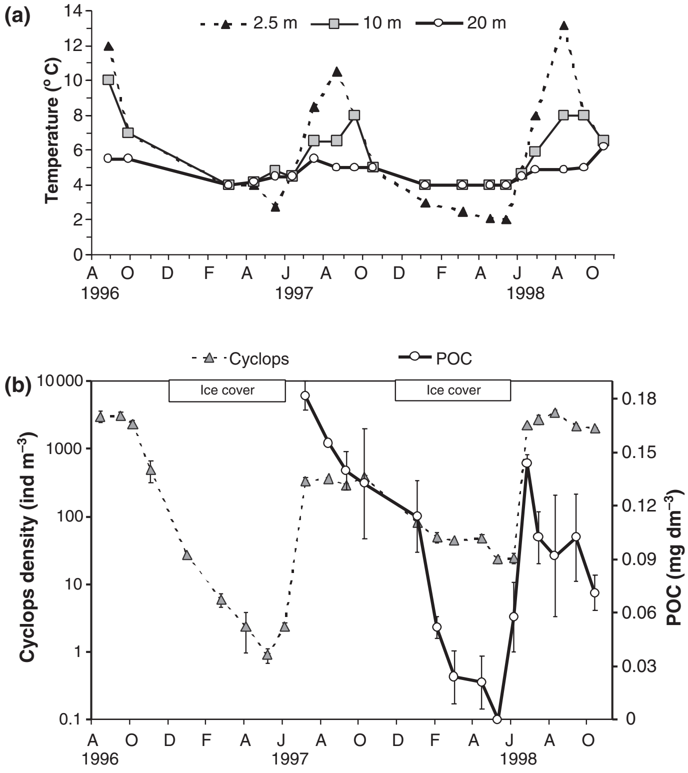

Fig. 2 Seasonal changes in (a) the water temperature in the upper pelagic zone of Czarny Staw and (b) the density of *Cyclops abyssorum* tatricus (copepodids and adult stage), the only predator of *Daphnia* in this lake (dotted line), and *Daphnia* food concentration based on POC analysis (solid line).

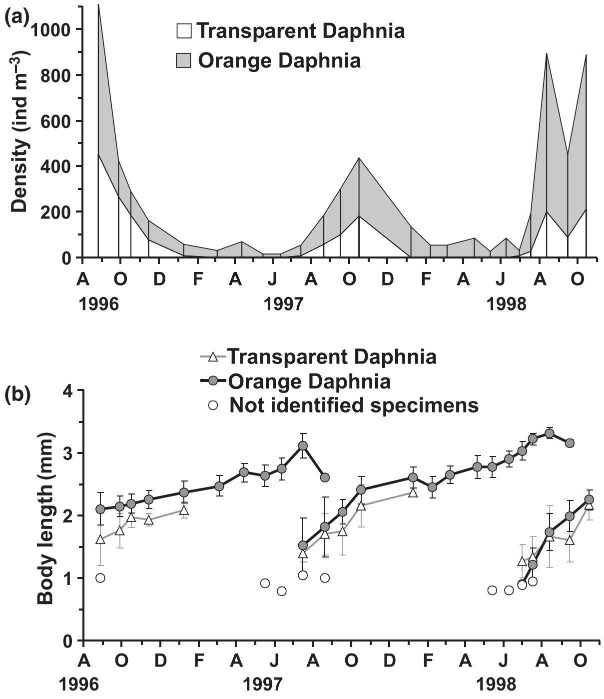

Fig. 3 Seasonal changes in (a) the densities of transparent and orange *Daphnia* morphotypes from Czarny Staw and (b) the body length (mean ± 1 SD) of orange and transparent *Daphnia* born in subsequent years.

May (Figs 3 & 4, Fig. S1). They most likely overwintered exclusively in the form of ephippial eggs. Floating ephippia were seen to hatch a few hours after being released from the ice cover (personal observation). The first transparent individuals formed subitaneous eggs in late June or early July and this continued until late September (Fig. 5). Ephippial females of transparent origin were recorded from August until the last sampling in November (no samples were taken in December due to thin ice cover). The most intense production of ephippial eggs was recorded in October; Minkiewicz (1911) observed the same in November and December. No males were recorded in the samples during the study period. Males were, however, observed in laboratory cultures of *Daphnia* from C. Staw (pers. obs.) and in some recent (2008) water samples from this lake (A. Bednarska, pers. comm.). The maximal densities of the transparent clone were recorded in August of 1996 and 1998, but in October of 1997. Following these peaks, the density decreased to the point of complete disappearance in February of the following years. Taken together these data indicate that the lifespan of transparent individuals was no longer than 7–8 months in this lake. Considering that analysis of population size structure indicated two or three generations of this clone per season, the lifespan of transparent individuals is likely to be considerably shorter than this. The last viable cohorts of transparent individuals appeared in August or September. Unfortunately, due to their size, the smallest individuals were difficult to classify based upon body colour alone. If it is assumed that the smallest individuals born in August comprised the animals later recorded in January, their maximum lifespan is likely to be 5–6 months.

In contrast to the transparent clone, orange individuals were active throughout the study period. Detailed examination of the size structure of orange *Daphnia* indicated a biennial lifespan. The first orange primiparous females appeared in March 1997 and May 1998, while newborn offspring (of likely orange origin) were observed in May in both years. Newborn orange individuals grew gradually but did not produce subitaneous eggs in their first season, despite the fact that their body size indicated potential maturity and body lipid levels appeared adequate. Instead, they accumulated rich lipid reserves which were gradually used up during the long winter (Fig. 5). These lipid reserves permitted the production of subitaneous eggs while under ice in the spring, before food conditions had improved in the lake. Orange individuals stored more lipid reserves than transparent specimens, with maximum lipid index values reaching 5 and 3, respectively (Fig. 5). About 2% (1996–97) and 11% (1997–98) of newborn orange *Daphnia* recorded in August survived the winter. The surviving females began forming subitaneous eggs under ice before food conditions had improved in the spring of their second season and continued reproduction until August–September, when they finally disappeared from the water column. Assuming May to be their month of birth and August/September of the following year to be their final month, the maximum lifespan of orange individuals cannot be longer than 15–16 months and may well be shorter. The last gravid orange females were recorded in August. If it is assumed that they survived until August/September of the following year, a conservative estimate of the maximum lifespan is 13–14 months, or two to three times greater than the transparent clone. Orange individuals did not

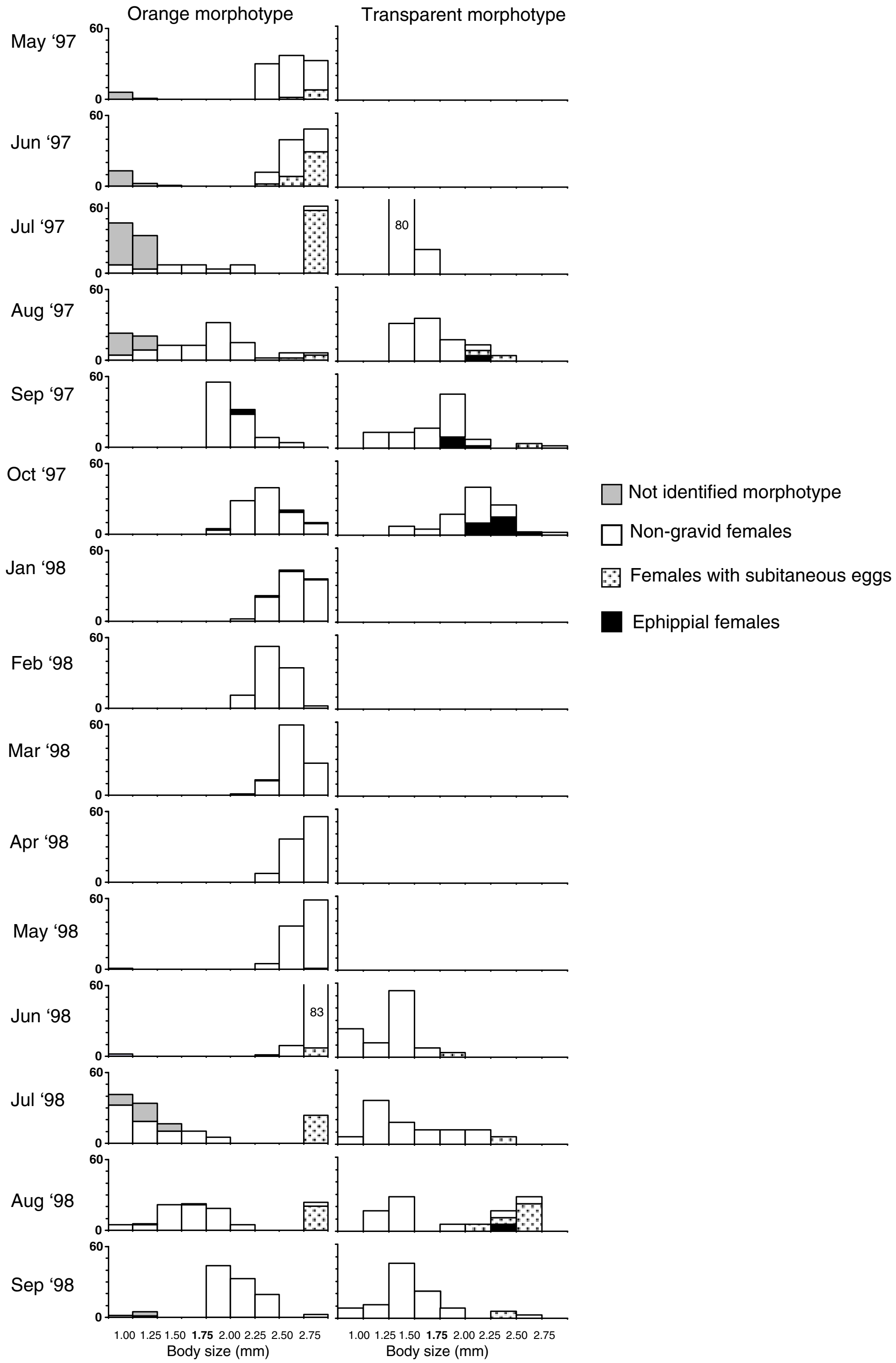

Fig. 4 Seasonal changes in the size structure of orange and transparent *Daphnia* morphotypes from Czarny Staw, May 1997–September 1998.

produce resting eggs or produced them only occasionally. Few orange ephippial females were recorded in the samples over the entire study period, which contrasts with the hundreds of transparent females observed in this state. Orange *Daphnia* were only seen to produce ephippial eggs in the first season, if ever

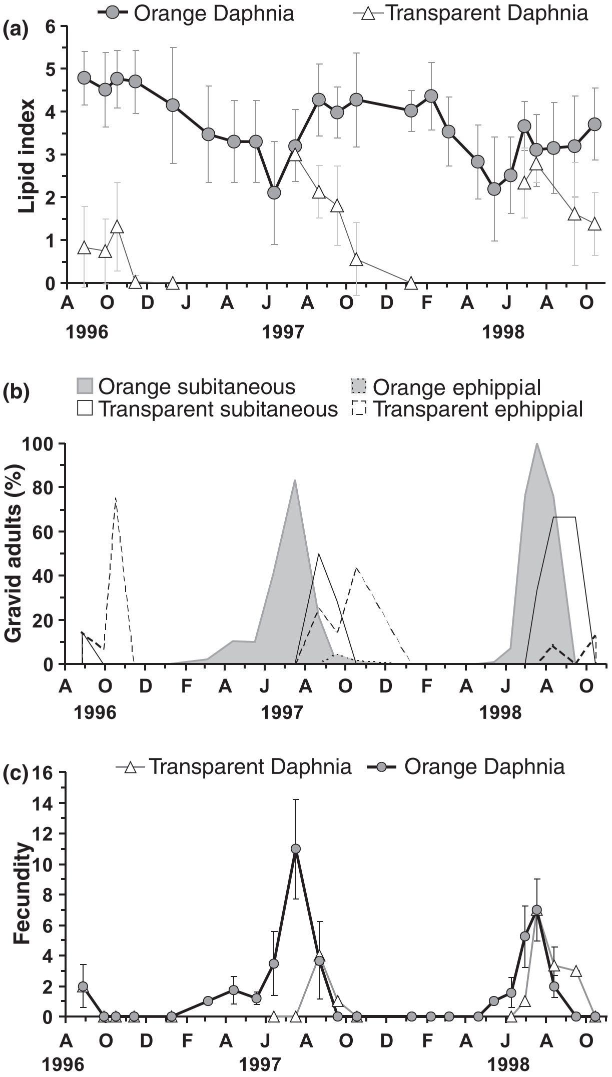

Fig. 5 Seasonal changes in (a) the lipid index (mean ± 1 SD), (b) the proportion of gravid adults and (c) the mean fecundity of orange and transparent *Daphnia* morphotypes from Czarny Staw (zero values indicate periods void of gravid females).

(Figs 4 & 5, Fig. S1). This indicates that orange individuals retained the ability to produce diapausing eggs yet rarely utilised it. Due to their greater lifespan and continuous growth, orange *Daphnia* attained a larger maximum body length than transparent animals (Fig. 4).

Discussion

The life strategies of the two *Daphnia* morphotypes inhabiting Czarny Staw differ from each other and from the typical lifestyle of Daphnidae in lowland habitats. The lack of males of both colour morphs in samples taken during the study period together with the earlier allozyme analysis of Cerny (1995), and the DNA analysis of recent samples (Markova *et al.*, 2007, M. Slusarczyk and J. Mergeay, unpubl. data), indicates the presence of only two clones of *Daphnia* in the lake and suggest that the *Daphnia* in Czarny Staw reproduce either exclusively or predominantly in a parthenogenetic manner. The transparent clone consists of short-lived animals (with lifetimes not exceeding 6 months in this cold lake) that complete their life cycle within a single season and survive winter exclusively as diapausing eggs, whereas the orange lineage includes biennial forms that remain active throughout the year. Newborn *Daphnia* of orange origin appear in the lake under ice cover as early as May. They grow and develop, accumulating rich lipid reserves, but do not reproduce in their first season, or do so only occasionally. They overwinter as active individuals and either do not produce ephippial eggs, or produce them occasionally in their first season. Instead they form subitaneous eggs in the following spring and summer. Orange individuals live for up to 13–16 months and disappear in September of their second season.

Is the exceptionally long lifespan of this clone within the normal physiological limits of other *Daphnia* or is it the result of selection for extended longevity? A recent laboratory study reported a maximum lifespan of 3 months for *D. pulicaria* (a close relative of the *Daphnia* in Czarny Staw) isolated from a permanent habitat, while the life duration of closely related *D. pulex* from temporary pools was only about half this (Dudycha, 2001). The reduced temperature and food concentration in the field compared to conditions used in the laboratory may partially explain the fourfold greater lifespan of orange *Daphnia* in Czarny Staw. However, this is not the full explanation since the long-lived orange forms reside in the same habitat as transparent *Daphnia* that live half as long or even less. Thus, the physiological lifespan of the orange form must have been extended in the course of natural selection.

Another unanswered question is why does this orange *Daphnia* not reproduce in its first season? Is it unable to or is it programmed not to do so? Reproduction in *Daphnia* is severely constrained by body size (Ebert, 1991) on the one hand and by food reserves (Tessier & Goulden, 1982) on the other. Females must reach a body size threshold before they can produce eggs. Orange *Daphnia* achieved larger body sizes than the egg-bearing transparent forms, so low body size was not the limiting factor for reproduction in their first season. Comparison of the lipid reserves of orange *Daphnia* in their second season (June–September), when they were forming subitaneous eggs, with those in the previous year (late summer, autumn or winter), indicates that sufficient reserves were present in the first season to be able to form eggs (Fig. 5). Moreover, when transferred from the lake to favourable food conditions in the laboratory, orange *Daphnia* did form subitaneous eggs in their first season (E. Rygielska and P. Maszczyk, pers. comm.). These findings indicate that orange *Daphnia* in Czarny Staw do postpone breeding until the second season.

Why do the two life strategies of *Daphnia* in Czarny Staw differ from the typical pattern seen in most daphnidae? The cyclically parthenogenetic mode of reproduction combined with short lifespan, typical in *Daphnia* from waterbodies of low latitudes and altitudes, requires favourable conditions for at least two generations to complete the life cycle and leave diapausing eggs. In lowland lakes the formation of diapausing eggs may be the only way to endure a long winter period when biotic (low food concentration and fish predation) and abiotic (oxygen depletion) threats limit the persistence of active forms. For some reason, *Daphnia* produce at most two diapausing eggs in a single clutch that hatch exclusively into females (Zaffagnini, 1987). Each year the first generation of cyclically parthenogenetic *Daphnia* that hatch from exephippial eggs is comprised solely of females. Ephippial eggs, which are formed in a sexual way, can thus not be completed before males born in the second generation can achieve maturity and fertilise them. In cold lakes or intermittent habitats, favourable seasons may be shorter than the two generations necessary to complete the life cycle of cyclically parthenogenetic forms. This time constraint and the imposed switch from cyclical to obligatory parthenogenesis, which allows *Daphnia* to complete its life cycle within a single generation (i.e. form ephippia by exephippial females) in cold polar habitats, has long been appreciated (Edmondson, 1955; Stross & Kangas, 1969).

The most favourable food conditions occur in Czarny Staw early in the season, shortly after the ice melts and before algae become depleted by the growing population of *Daphnia*. According to the experimental studies of Kreutzer (1999), older *Daphnia* can survive at lower food concentrations than young specimens and thus are likely to deplete food below a tolerable level for the further. By September, the production of active *Daphnia* had ceased in Czarny Staw; newborns were not recorded in the lake despite the finding that mature females were still forming subitaneous eggs. Either the small *Daphnia* had starved to death due to poor food conditions or they were exterminated by the ample population of invertebrate predators such as *Cyclops abyssorrum* tatricus, as suggested by Gliwicz *et al.* (2001). The three-month period of relatively favourable food conditions yet low water temperatures in Czarny Staw (June–August) appeared to be just sufficient for the production of two to three generations (first exephippial, second derived from subitaneous eggs of exephippial females) of transparent *Daphnia* (indicated by the decrease in mean body size when a new generation appeared each season). This short period was quite close to the threshold for the persistence of cyclically parthenogenetic forms. However, it is likely that favourable periods for reproduction may have been shorter in colder years (e.g. during the Little Ice Age in past centuries) in Czarny Staw, or in habitats at higher altitudes or latitudes from where *Daphnia* may have immigrated to Czarny Staw. Shortened favourable periods for reproduction in colder years or colder habitats may have excluded cyclically parthenogenetic *Daphnia* of short lifespan from the high mountain habitats.

As mentioned above, the two colour morphotypes of *Daphnia* in Czarny Staw solve the challenge of this short time 'window' of favourable conditions for the development of young specimens in two different ways. They either switch to obligatory parthenogenesis, like many other *Daphnia* in polar (Edmondson, 1955; Hobaek & Weider, 1999; Weider *et al.*, 1999) or high mountain habitats (Aguilera *et al.*, 2007), which allows them to produce diapausing eggs in a single generation (transparent *Daphnia*), or they postpone reproduction and senescence until food conditions improve again in the following season (orange *Daphnia*). In high mountain, permanent lakes that are devoid of planktivorous fish – the key biotic cause of *Daphnia* mortality in lowland lakes – selection against older forms is relaxed while the survival of young specimens with low tolerance to poor food conditions is reduced. This is likely to have led to the evolution of postponed reproduction (reproductive diapause) and a biennial life cycle (extended lifespan) in orange *Daphnia* in Czarny Staw as well as *Daphnia umbra* in Stasjonsdammen recently reported by Larsson & Wathne (2006). Both strategies revealed by transparent and orange *Daphnia* appear advantageous in permanent habitats with a short time window in which conditions are favourable for reproduction. A mixed strategy would be maladaptive in Czarny Staw. Reproduction in the first season might not allow orange *Daphnia* to accumulate sufficient lipid reserves to survive the long winter. On the other hand, extended lifespan in transparent *Daphnia* would demand lipid reserves necessary to form ephippial eggs capable of surviving the long winter. The complete abandonment of diapausing egg formation by long-lived orange *Daphnia* would be a risky strategy, as a single season with low survival and failed reproduction could terminate a population that did not leave a persistent bank of diapausing eggs. This is probably the reason why orange *Daphnia* were found to occasionally produce diapausing eggs in Czarny Staw. A second possible reason for retaining ephippia formation in orange *Daphnia* is that dispersal of ephippial eggs seems to be a major way in which isolated freshwater habitats are colonised (Havel & Shurin, 2004, De Meester *et al*., 2002; Pietrzak & Slusarczyk, 2006). Without diapausing eggs, migration between isolated sites may be less likely, if possible at all. While ephippial eggs are most likely produced in an asexual way by transparent *Daphnia* it is uncertain in what way they are formed by orange females. It should be noted that extended lifespan does not necessary imply the loss of sexual reproduction by orange *Daphnia*. The recent microsatellite analysis of ephippial eggs of the orange *Daphnia* indicates that they are formed most likely in asexual way (M. Slusarczyk and J. Mergeay, unpubl. data).

The two colour morphs of *Daphnia* seem to have coexisted in Czarny Staw for a long time. Both appear to have been present in this lake a century ago (Minkiewicz, 1911). One unanswered question is why has one of these two morphotypes of contrasting life strategies not become dominant? Preliminary data indicate that this may be due to the existence of spatial segregation between the two. While transparent individuals reside in subsurface warmer waters during the summer, most probably to speed up their development and complete the life cycle within a single season, orange forms stay in cooler deeper regions of Czarny Staw (unpubl. data), presumably to slow down metabolic activity what may help them to survive till the next season and initiate reproduction when food conditions improve again. Spatial segregation may have reduced resource competition between the two lineages and allowed their coexistence in Czarny Staw.

A final intriguing question is what is the function of the orange pigmentation in *Daphnia*? This colour is most probably due to carotenoids, and three potential functions of these compounds in aquatic crustaceans have been proposed: photoprotection (especially against UV radiation; Hairston, 1980), energy storage (Ringelberg, 1980) and antioxidant protection (Cornet, Biard & Moret, 2007). The first of these hypothetical functions is repeatedly claimed despite little supporting evidence. The orange *Daphnia* in Czarny Staw reside deeper in the water column than the transparent clone, which does not fit the UV protection hypothesis. Furthermore, in a laboratory test, orange *Daphnia* appeared more vulnerable to UV radiation than transparent individuals (E. Rygielska and M. Slusarczyk, unpubl. data). The second potential function of carotenoids as an energy storage substrate has not been tested, so cannot be excluded. It would be useful to know if there is any advantage for overwintering individuals in storing energy in carotenoids rather than lipids. The third and most intriguing hypothetical function of carotenoids is their potential role as antioxidants. Does the orange *Daphnia* owe its long lifespan to this well recognised property of carotenoids or is the association of this pigment with longevity just coincidental? Further investigation is required to answer this question.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Seasonal changes in the size structure of orange and transparent *Daphnia* morphotypes from Czarny Staw, from August 1996 to October 1998.

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To sink or float: the fate of dormant offspring is determined by maternal behaviour in *Daphnia*

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SUMMARY

1. As the ephippia (chitinous shells enclosing diapausing eggs) of pelagic crustaceans of the genus *Daphnia* have been occasionally reported to float at the water surface, we considered that this might be an adaptation promoting their passive dispersal. We investigated the mechanisms by which ephippia appear at the water surface.
2. While field surveys revealed that floating *Daphnia* ephippia are often numerous in various freshwater habitats, laboratory tests showed that newly formed ephippia are not buoyant initially. Once transferred to the surface by whatever means, however, they may remain there due either to surface tension or gas absorption.
3. Video recordings showed that all ephippia at the water surface in laboratory vessels were shed there by ephippial females when moulting (despite the attendant risk of exposure to UV radiation). This implies that the moulting behaviour of female *Daphnia* may determine the fate of their dormant offspring, predetermining whether they remain in the natal environment (when the ephippium is released into the water column) or disperse (when it is deposited at the water surface).
4. Our findings reveal a potential mechanism underlying the high dispersal capacity of freshwater cladocerans inhabiting island-like aquatic habitats.

Keywords: Daphnia, diapause, dispersal, ephippium, vector

Introduction

The production of dormant stages is a widespread adaptation among organisms inhabiting periodically deteriorating environments. The resistance of dormant stages to unfavourable conditions not only allows them to re-colonize the native habitat after conditions have recovered (dispersal in time), but may also facilitate their passive transport to new locations (dispersal in space) (Venable & Lawlor, 1980; Fryer, 1996; Gyllström & Hansson, 2004). For immobile (e.g. plants, sessile animals) or mobile organisms inhabiting isolated sites (e.g. parasites, animals on islands), the passive dispersal of dormant stages may be the only feasible way to colonize new habitats.

This seems also to be the case for pelagic crustaceans of the genus *Daphnia,* which are ubiquitous inhabitants of lakes and ponds (Fernando, Paggi & Rajapaska, 1987; Hrbacek, 1987) and are fast colonizers of new habitats (Louette & De Meester, 2005) despite lacking mechanisms for active dispersal. In their life cycle, they facultatively produce diapausing eggs enclosed in a chitinous shell, called an ephippium, formed from the dorsal part of the carapace and which enhances the resistance of diapausing eggs to unfavourable conditions. Another function of the ephippial shell that is less obvious is that it may facilitate the dispersal of diapausing eggs. It is widely appreciated that *Daphnia* owes its high dispersal capacity to diapausing eggs, which are passively transported by biotic (e.g. humans, Johnson, Ricciardi & Carlton, 2001; waterfowl and other amphibious animals, Figuerola & Green, 2002; Figuerola, Green & Michot, 2005) or abiotic vectors (e.g. wind as suggested by Brendonck & Riddoch, 1999; or in outflows,

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Michels *et al.*, 2001). However, additional specific features of ephippial shells (their hydrophobic nature or spiny surface structure), apart from providing mechanical protection, may also aid the passive dispersal of diapausing eggs.

Daphnia shed ephippia into the surrounding water during cyclical moulting. If the ephippium sinks to the bottom, diapausing eggs have to wait for favourable period in their natal habitat. Large numbers of ephippial eggs may be found in sediments (Carvalho & Wolf, 1989), where they may remain viable for years or even decades (Cáceres, 1998) ready to hatch upon the onset of suitable conditions. For reasons that remain unclear, some cladocerans glue ephippia to submerged plants (Brendonck & De Meester, 2003). Ephippia have also occasionally been reported to form vast accumulations of dormant propagules along surf lines at the water surface or on the shore (Fryer, 1996; Wetzel, 2001; Kerfoot *et al.*, 2004). The common occurrence of ephippia at the water surface of freshwater habitats and their potentially significant role for *Daphnia* dispersal imply that this phenomenon may not be purely accidental but may have adaptive value. While the significant role of floating ephippia in *Daphnia* dispersal has been considered recently (Pietrzak & Ślusarczyk, 2006; Cáceres, Christoff & Boeing, 2007), the mechanism by which they appear at the water surface has not yet been investigated.

Here we considered two potential mechanisms by which ephippia may appear at the water surface. Potentially, all ephippia might be shed in the water column and sink or float according to their relative densities determined by the content of additives such as gas or lipids. Alternatively, ephippia might be trapped in the surface film (despite their negative buoyancy) if deposited there by their mothers. To test these possibilities we first assessed the buoyancy of freshly deposited ephippia. Second, we investigated whether the females deposited their ephippia at the surface. We discuss the potential functions of the 'intentional' deposition of ephippia at the water surface by females.

Methods

Field survey

Densities of ephippial females in the water column and ephippia floating at the water surface were determined in the central zone of 15 lakes and ponds in north-east Poland and in a single lake (Brome) in the south-east of Canada (see Table 1), at times when ephippia were being produced. The samples in Poland were taken in October (2005) and these from lake Brome in June (2007). In each lake, except the two shallowest ponds, three quantitative plankton samples were collected with a funnel shaped plankton net (of 14 cm wide circular opening, 150 μm mesh size), that was towed from the bottom to the water surface. In all lakes and ponds three quantitative samples were collected with a neuston net (a modified plankton net of 15 cm wide and 20 cm high rectangular opening, 150 μm mesh size) half-submerged during horizontal 100-m long tows. The samples were preserved in the field in 4% formaldehyde solution and analysed in the laboratory thereafter. Densities of ephippial females in the water column and floating ephippia at the water surface were estimated based on these samples.

Indirect test of physical forces that might keep ephippia at the water surface

This test was performed in 125 mL cylindrical glass vials (25 cm high, 2.5 cm wide) filled with Artificial Daphnia Medium ('ADaM', see Klüttgen *et al.*, 1994). The medium was supplied with the algae *Scenedesmus obliquus* Turp used as food at a concentration of 0.5 mg C L^{-1}. Four *Daphnia* species, all originating from waterbodies sampled in the field study, were tested separately. Three species originated from a shallow, temporary pond located in Warsaw, Poland (Waw, see Table 1): large bodied *D. magna* Strauss, medium size *D. pulex* de Geer and small daphnia from the *D. longispina* species complex. The fourth species, medium sized *D. pulicaria* Forbes, originated from the permanent lake Brome in Canada. *Daphnia pulex* and *D. pulicaria* are closely related species and belong to the common species complex called *D. pulex*. Ephippial females found in the field were transferred to the laboratory and randomly assigned to experimental vials (12 females per vial) in four treatments, within 6 h of their collection. The experiment was conducted under constant illumination (approximate intensity 2.5 μmol m^{-2} s^{-1}) at 23 °C, until all females had shed their ephippium (i.e. within 48 h).

In the first 'control' treatment conditions remained unmodified. In the second 'UV' treatment *Daphnia*

Table 1 Mean density of floating ephippia and ephippial females in the water column at the centre of lakes and ponds in Poland in autumn 2005. The only Canadian lake (Brome) was sampled in late spring 2007

Density of floating ephippia (m^{-2})	Density of ephippial females in the water column (m^{-2})	Species	Lake	Area (ha)	Max depth (m)	Latitude	Longitude
0.1	35	L	Przystajne	32	16	N54°13.29′	E22°40.11′
0.7	0	L	Roś	1888	31	N53°40.09′	E21°55.31′
5	37	L	Jorzec	42	12	N53°83.65′	E21°51.18′
5	8	L	Kociołek	15	13	N54°03.03′	E22°20.08′
9	34	L	Hańcza	311	109	N54°15.04′	E22°48.22′
10	33	L	Długie	36	12	N54°05.75′	E22°31.79′
11	34	L	Sołtmany	180	13	N54°37.51′	E22°01.21′
15	34	L	Białe	132	52	N54°19.65′	E22°65.47′
17	298	L	Zyzdrój	214	14	N53*39.37′	E21*17.40′
55	12	L	Garbaś	153	48	N54°08.07′	E22°37.51′
58	130	L	Szelment	356	45	N54°14.19′	E22°58.70′
60	150	L	Ożewo	55	56	N54°08.84′	E22°48.81′
100	103	L	Okmin	114	40	N54°09.26′	E22°49.87′
653	na	L	Malse	2.7	1.5	N53°36.19′	E21°36.83′
880	2860	P	Brome	1450	13	N45°15.04′	W72°30.50′
1076	na	M, P, L	Waw	0.2	0.7	N52°13.69′	E21°01.95′

L – *D. longispina*, P – *D. pulex*, M – *D. magna*. na – data not available.

were exposed to constant UV radiation (generated by a 40 W Phillips Cleo UV fluorescent bulb, approximate intensity 2.5 μmol m^{-2} s^{-1}) at the water surface. UV light was applied to discourage experimental females from staying close to the surface and prevent accidental shedding of ephippia there. In the third 'net' treatment, the upper part of experimental vials was occluded with nylon mesh positioned 1 cm below the surface, in order to prevent any contact of ephippial females with the surface film. In the fourth 'detergent' treatment, the surface tension of water in the open vials was reduced by adding to each vial 300 μg of non-toxic detergent, cetyl alcohol, commonly used in *Daphnia* cultures. In this experiment, if ephippia floated due to positive buoyancy, we should have observed them in the upper part of all experimental vials, regardless of the treatment. If they floated as a result of deposition at the water surface by the parent, we should have observed them at the surface in the open vials only ('control' and 'UV'). Each treatment had seven replicates in case of *D. longispina* and *D. magna* and 11 replicates in case of *D. pulex* and *D. pulicaria*. Within 48 h of their deposition, the buoyancy of ephippia found at the water surface was tested by pushing them below the surface film while ensuring that any attached air bubbles were dislodged.

Non-parametric tests were used for statistical analysis of the experimental results. Mann–Whitney test with Bonferroni correction was used for pair-wise comparisons of the parameters tested. Kruskal–Wallis analysis with *post hoc* Dunn's Multiple Comparison Test was used when more than two groups were compared simultaneously.

Direct test of the mechanism by which ephippia appear at the water surface

Daphnia of three common species groups: *D. longispina, D. pulex* and *D. magna,* all originating from the shallow temporary pond, were tested. The animals were cultured for several weeks prior to the experiment and ephippial production was stimulated by crowding.

This test, aiming at determining the way ephippia appear at the water surface, was conducted at 22 °C in 60 cm tall and 7 cm wide aquaria filled with artificial water medium (ADaM) with the addition of food (the green alga *Scenedesmus obliquus*) at a concentration of 0.3 mg C L^{-1}. Containers were exposed from above to UV radiation of the same source and intensity as in the previous tests. *Daphnia* were placed individually in 60-cm long and 1-cm wide glass tubes positioned vertically in the aquaria (four tubes in each aquarium). The top 10-cm water layer was continuously observed with a video recorder and the way ephippia appeared at the water surface was analysed thereafter from the moving pictures. Based on the proportion of the time spent at the water surface by experimental females in relation to the total time of the recording, we tested whether ephippial females deposited ephippia at the water surface accidentally or actively.

Results

Field survey

Floating ephippia were widespread and common. During our lake survey in autumn 2005 we recorded floating ephippia at various densities and intensities (in relation to density of ephippial females in the water column) in all (16) lakes and ponds surveyed (Table 1). The highest densities of floating ephippia were recorded in two contrastingly different habitats: a temporary Warsaw pond in autumn 2005 ($1076\ \mathrm{m}^{-2}$) and in the permanent Lake Brome in early June 2007 ($880\ \mathrm{m}^{-2}$), both of which were sources for experimental *Daphnia* populations. We did not test, however, the differences in densities of floating ephippia between lakes as they were likely to be determined by environmental factors not controlled in this study (e.g. wind, waves and rain action).

Indirect test of physical forces keeping ephippia at the water surface

We found different proportions of ephippia floating in the upper part of experimental vials in different treatments and for different species (Fig. 1). All ephippia were found at the bottom in the 'net' and 'detergent' treatments, where access to the surface film was denied or surface tension reduced. In the control treatment, *D. longispina*, *D. pulicaria* and *D. pulex* left more ephippia at the water surface than *D. magna* (*post hoc* Dunn's Multiple Comparison Tests, $P < 0.05$, after a significant difference between species was found using a Kruskal–Wallis test, $F_{3,36} = 18$, $P < 0.0005$). The first three species, however, did not differ between each other in this parameter. Exposure to UV significantly reduced the proportion of ephippia deposited at the surface compared to the control treatment in three of four tested species: *D. pulex* ($U_{1,11} = 6$, $P < 0.0001$), *D. pulicaria* ($U_{1,11} = 20.5$, $P < 0.01$) and *D. magna* ($U_{1,7} = 3.5$, $P < 0.005$) (pairwise comparisons in Mann–Whitney tests with Bonferroni correction).

Most of the ephippia that were found at the water surface in the four species tested were negatively buoyant and sunk when pushed below the water surface (Fig.1). They were clearly kept at the water surface by surface tension forces. Some of the floating ephippia did not sink even when pushed below the water surface and remained at the water surface thanks to gas bubbles lodged either inside the double wall of the ephippial 'shell', or between the two parts of the shell. The highest proportion of positively buoyant ephippia were recorded in *D. longispina* (over 20%), and no positively buoyant ephippia were found in *D. magna*.

Direct test of the mechanism of ephippia appearance at the water surface

Video recordings of ephippial females exposed to the surface threat of UV radiation revealed that all ephippia found floating had been actively deposited at the water surface by the mother while moulting. Some of the ephippial females (different proportions in different species) approached the surface, broke the surface film, shed the ephippium at the water surface within a few tens of seconds and moved down promptly thereafter (Fig. 2). The highest proportion of experimental females depositing ephippia at the water surface was observed, as in previous tests, in *D. longispina* (31 out of 32 individuals). Fewer females of *D. pulex* left ephippia at the surface (39 out of 73 individuals, $\chi^2 = 18.9$, d.f. = 1, $P < 0.0001$), while none of *D. magna* (out of 82 individuals) approached the surface nor left ephippia there during this trial. Females that left ephippia on the bottom (117 of all 187 tested animals) either did not approach the surface while moulting (98%) or did not manage to leave the ephippium there (2%).

The brief appearance of ephippial females at the water surface compared to total observation time (with the relative time ratio 1 : 243, in *D. longispina* and 1 : 1100 in *D. pulex*) indicates active deposition of ephippia at the surface film in two of three species of *Daphnia* tested.

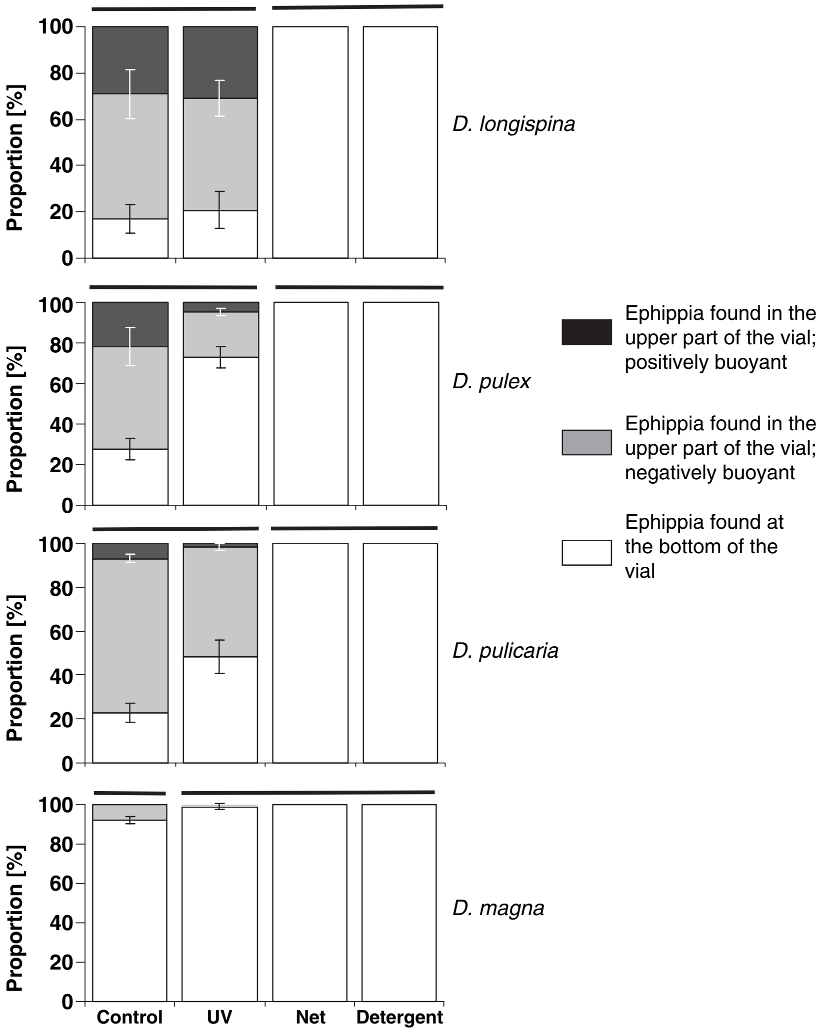

Fig. 1 Mean ratio of floating ephippia versus ephippia recorded at the bottom (standard error indicated by black error bars) in four *Daphnia* species, in four types of experimental vials: 'control' – open at the top; 'UV' – open at the top and exposed to UV radiation; 'net' – with net installed below the water surface; and 'detergent' – open at the top, with detergent added. White error bars indicate the standard error of the proportion of positively buoyant ephippia among those found at the surface. Horizontal lines above the bars indicate that treatments in each species tested for the proportion of floating ephippia were not significantly different (*post hoc* Dunn's Multiple Comparison Tests, $P < 0.05$).

Discussion

Although reports of the success with passive dispersal and establishment in a new favourable location seem relatively limited (De Meester *et al.*, 2002; Bohonak & Jenkins, 2003), many aquatic organisms have propagules apparently adapted for dispersal (Bilton, Freeland & Okamura, 2001). Ephippial eggs can resist unfavourable conditions for long periods, making them ideal for passive dispersal between aquatic 'islands' scattered across an inhospitable terrestrial landscape (Fryer, 1996; Gyllström & Hansson, 2004). Such passive dispersal of dormant stages may potentially be promoted in various ways. However, since most vectors of overland dispersal operate at the surface of freshwaters, deposition of propagules at the surface might enhance their chances of travelling to new locations (Pietrzak & Ślusarczyk, 2006). Indeed, Davison (1969) was the first to report the release of ephippia by pelagic *Daphnia* at the water surface, though this anecdotal observation has since been overlooked, the potential adaptive value going unrecognized for all this time. Our research is now able to confirm Davison's (1969) observations, and to challenge the view that the appearance of ephippia at the water surface is a mere accident.

Our field survey showed the widespread occurrence of floating ephippia at times when they were being produced, even if it could not identify mechanism by which they appeared at the water surface. While the highest density of floating ephippia was observed in a shallow pond, it is not known if this reflects a higher intensity of ephippia deposition at the water surface or simply better protection from the wind, which would carry ephippia toward the shore in more exposed lakes.

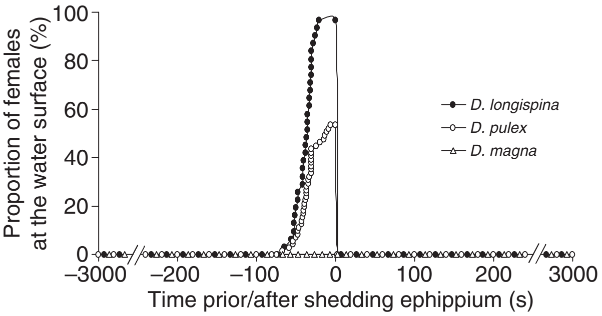

Fig. 2 Timing of appearance at the surface of experimental *Daphnia* before/after shedding ephippium (indicated by 0 on the *x*-axis) when exposed to UV radiation.

Equally, our laboratory tests refuted the possibility raised in other studies (Cáceres *et al.*, 2007) that *Daphnia* ephippia are positively buoyant. Had that been the case, floating ephippia would have been found in all treatments in the first experiment (Fig. 1). Rather, we found floating ephippia only in those experimental vials offering females unrestricted access to the surface. This implies that ephippia were not initially buoyant, yet found their way to the surface through maternal behaviour or some other mechanism. Video recordings confirmed these speculations, demonstrating that a proportion of the ephippial females (varying from species to species) release ephippia directly at the water surface while moulting. Moreover, our data show that this is not accidental. *Daphnia* seems to exploit the surface tension to keep non-buoyant ephippia at the air-water interface. The hydrophobic structure of the ephippial shell may facilitate its binding to the water surface. Under natural conditions, the persistence of ephippia in the surface film is unlikely to be assured by these weak forces easily counteracted by external agents like wind or rain. In addition, natural substances accumulating in the surface film may reduce surface tension significantly (Goldacre, 1949) and affect the proportions of floating ephippia like the detergent in our study did (Fig. 1).

Although freshly released ephippia are apparently non-buoyant, some may become buoyant having come into contact with the water surface. Our trials revealed the presence of gas bubbles, either between shells or within the double wall of the buoyant ephippia. Since bubbles in ephippia were only reported from vials offering experimental females unrestricted access to the air-water interface, they probably comprised atmospheric air that shells captured after contact with the surface. Air absorption by ephippial shells might be of adaptive value, if it kept ephippia at the water surface and furthered dispersal.

The release of ephippia at the water surface puts both parental females and ephippial eggs at increased risk, for instance of enhanced exposure to UV radiation or predation by waterfowl (Gardarsson & Einarsson, 2002) or fish (Mellors, 1975). Moreover, we observed females becoming trapped by surface tension at the water surface as they released ephippia. While active individuals are indeed vulnerable at the surface, ephippial eggs are found to be highly resistant to many of the risks associated with the surface. The ephippial eggs of *Daphnia* are believed to tolerate high levels of UV radiation, drying and freezing, and they may even survive consumption by predators if not mechanically damaged (Mellors, 1975). The fact that *Daphnia* mothers are willing to run the risk of depositing of ephippia at the water surface suggests that the phenomenon has an adaptive function. Furthermore, the risks may be diminished in nature if the females shed ephippia at night (as is indicated by our preliminary unpublished data).

The smallest species, *D. longispina*, deposited the greatest proportion of ephippia at the surface among the four species studied. Moreover, this was the only species that left ephippia at the surface regardless of whether or not it was exposed to the threat of UV radiation (Fig. 1). Finally, *D. longispina* left the highest proportion of positively buoyant ephippia. The reasons for these species differences are unclear.

While ephippia deposited at the water surface may be the most likely to disperse, it is not clear whether dispersal operates mainly within or between aquatic habitats. There could be adaptive value in either case. Though risky, dispersal between environments may avoid unfavourable conditions, facilitates the colonization of vacant habitats (Levin, Cohen & Hastings, 1984) and reduces kin competition (Hamilton & May, 1977). On the other hand, deposition of ephippia at the water surface may facilitate passive transport by currents to the shallows of the natal habitat. This may help avoid the sedimentation of ephippia in the profundal of deep lakes, where reliable hatching cues like light or temperature changes may be absent (Cáceres, 1998). Moreover, in temporary ponds the deposition of ephippia in the shallows might promote hatching only after the pond is completely refilled, thus assuring persistence of the aquatic habitat, the mechanism known in fairy shrimps inhabiting ephemeral pools (Hildrew, 1985). The ultimate reasons for the phenomenon will only be determined by further investigation, however.

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Short review

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THE FATE OF THE EPHIPPIA – *DAPHNIA* DISPERSAL IN TIME AND SPACE

ABSTRACT: When conditions deteriorate, freshwater cladocerans from the genus *Daphnia* switch to production of diapausing eggs encased in chitinous shells called ephippia. Ephippia may serve for temporal escape and recolonisation of the habitat or for geographical dispersal and colonisation of other habitats. While dormancy and dispersal of ephippia have been well studied, initial factors determining the fate of the ephippia have remained unknown.

We first consider the fate of an ephippium that sinks to the bottom of the water body, where it enriches the egg bank present in the sediments. We then discuss the potential and evidence for dispersal of ephippia, which is probably facilitated by their flotation on the water surface. Finally, we concentrate on the moment of the release of the ephippium showing a likely mechanism that determines its further fate – temporal or spatial dispersal – and we demonstrate it to be accomplished through maternal behaviour.

KEY WORDS: *Daphnia*, ephippium, diapause, dispersal, maternal influence

1. INTRODUCTION

For annual plants, only two strategies are possible when unsuitable conditions arise: escape in time or escape in space. Annuals may escape in time by fractional or delayed seed germination or they may escape spatially to new patches via seed dispersal (Venable and Lawlor 1980). Whether a seed is destined for dispersal or not is maternally determined, either by seed morphology or morphology of the mother plant (Venable and Brown 1988, Donohue 1998). Similarities of these two escape mechanisms and maternal determination may be found in the animal kingdom.

Freshwater planktonic cladocerans (Crustacea, Branchiopoda) produce dormant eggs which may serve these two functions: they provide a mode of avoiding harsh periods by seeking refuge in time and they get dispersed between non-contiguous bodies of water. These diapausing eggs are encased in protective chitinous shells called ephippia. Ultimate reasons for diapause in cladocerans from the genus *Daphnia*, proximate factors inducing ephippia production, their resistance to various environmental conditions, as well as ecological and evolutionary consequences of their production, have been well studied (Bohonak and Jenkins 2003, Brendonck and De Meester 2003, Gyllström and Hansson 2004, for recent reviews). However, factors determining their fate, destining them for

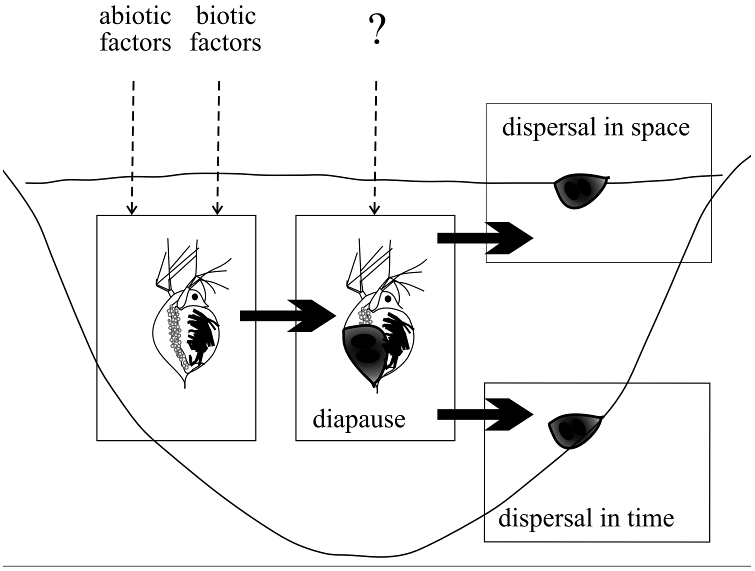

Fig. 1. Part of the *Daphnia* life cycle: from active to dormant stage (ephippium) and its further fate. Ephippia sinking to the sediments serve as a means of temporal dispersal and ephippia floating at the water surface as a means of spatial dispersal.

prolonged dormancy or for dispersal, have not yet been identified.

We suggest that the overriding factor determining whether ephippial eggs would serve as a means of temporal escape or of geographical dispersal might be the place of their deposition, i.e. sinking to the sediments or floating on the water surface (Fig. 1). We observed that this might be determined by the mother's behaviour.

2. SINKING EPHIPPIA

The active part of a *Daphnia* population dwells in the pelagial zone, whereas large numbers of ephippia are found in the sediments. Therefore, most ephippia are believed to be shed in the water column and sink directly to the bottom of water bodies, where they form egg banks. Such egg banks in the sediments are analogous to seed banks of plants in the ground and may reach densities of tens of thousands of ephippia per square metre (Cáceres 1998, Vandekerkhove *et al.* 2005a). The horizontal distributions of ephippia in the lake beds are reported to be concentrated in deep waters (Carvalho and Wolf 1989). This is often explained by higher deposition of ephippia in places where the overlying water column contains a larger population (Brendonck and De Meester 2003).

Production of ephippia marks the beginning of what can be thought of as a time travel (Hairston 1998). Resting eggs of *Daphnia* will resume activity when conditions improve at a later, more favourable time, most likely in spring (Cáceres and Schwalbach 2001). Hatching usually takes place at the onset of the growing season when the opportunity for growth and reproduction appears, and before the lake stratifies, when environmental cues such as light or rise in temperature are cut off (Wolf and Carvalho 1989). However, not all ephippial eggs resume development at the first suitable occasion. Some remain dormant for longer periods, thus possibly "hedging bets" against future unpredictable catastrophes (Cáceres and Tessier 2003).

Theory predicts the fraction of ephippia hatching each year, but the observed emergence in the field is well below the predicted values (Cáceres 1998). Reduced light penetration, low oxygen levels and consistently low temperatures in deeper waters can prolong diapause (Carvalho and Wolf

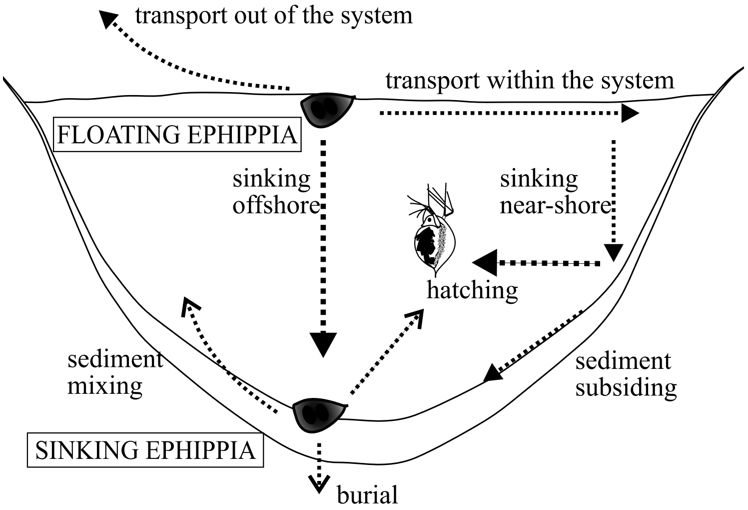

Fig. 2. Possible fate of ephippia sinking to the sediments and floating on water surface (see Cáceres and Hairston 1998, Gyllström and Hansson 2004 for comparison).

1989), however this might not be adaptive. In addition, there is a risk of rapidly being buried in deeper strata with submergence into the sediment. In eutrophic lakes sedimentation rates may be as high as several centimetres per year, whereas hatching only takes place near the sediment-water interface (Cáceres 1998). As Cáceres and Tessier (2003) point out, the majority of the egg bank is likely never to receive the hatching stimulus in most lakes.

Burial in the sediments, however, does not necessarily preclude the ephippium from hatching later (Fig. 2). Chances for reappearance of the ephippium at the sediment surface decrease with its burial depth, but sediment mixing by bioturbation and water movements may bring it back close to the surface or even to the water column, and while deep sediment mixing may be rare, viable ephippia have been found in sediments dated over one hundred years old (Cáceres 1998). Whether staying viable in diapause for decades is an adaptation to unpredictable environmental conditions or a consequence of egg burial (Hairston *et al.* 1995), sinking to the sediments creates opportunities for temporal but not for spatial dispersal.

3. FLOATING EPHIPPIA

Cáceres (1998) reported that estimates of annual production of ephippia at times were more than double the numbers of those effectively deposited into the sediments and hatching annually. The missing ephippia could probably be found floating at the water surface or attached to vegetation, both of which have been reported anecdotally in the literature. According to Brendonck and De Meester (2003) these ephippia should be considered a regular part of the egg bank despite the fact that their fates may be considerably different from that of ephippia deposited in the sediments. Ephippia deposited in deep spots have a low chance of receiving hatching stimuli and production of floating ephippia might enable their passive surface transport towards the shores. Sinking to the sediments near-shore may be advantageous because of the exposure to more pronounced environmental cues absent in deep waters and thus the ability to hatch at the appropriate time for successful recolonisation of the habitat. It has been claimed already that hatching takes place mainly in shallower waters (Wolf and Carvalho 1989) and some authors have suggested that the littoral zone may play an important role in the recruitment of *Daphnia* in large lake populations (Weider et al. 1997, Jankowski 2003). Furthermore, if they are buried near-shore, they are more likely to be brought back to the sediment surface through mixing.

On the other hand, deposition of ephippia at the water surface may be a mechanism promoting *Daphnia* dispersal between lakes. By migrating to new habitats *Daphnia* might a) avoid local deterioration of environmental conditions, b) reduce parent-offspring conflict (Ellner 1986) or c) simply spread its genes. Since most animate (birds, amphibious animals, humans) and inanimate (wind, outflow) potential vectors of ephippia dispersal operate in the surface or sub-surface zone of freshwater habitats, ephippia floating at the water surface have facilitated contact with these vectors.

Whether ephippia do get dispersed frequently has been often discussed. Discrepancies between the high potential for dispersal and low levels of gene flow inferred from genetic variation between populations have given rise to much controversy and promoted a debate on the ecological and evolutionary significance of dispersal in freshwater zooplankton (Bohonak and Jenkins 2003, Havel and Shurin 2004, for critical reviews). Recent studies give evidence for ongoing effective dispersal of cladocerans (Figuerola *et al.* 2005, Louette and De Meester 2005). High colonization rates observed for newly created habitats show ephippia move frequently between water bodies (Vanderkhove *et al.* 2005b), but the establishment success may be constrained by local interactions, as predicted by De Meester *et al.* (2002).

Whether aimed at within lake or between lakes dispersal, ephippia staying at the water surface, as well as those transported overland, might be exposed to environmental factors not present in deep waters. Ephippia show resistance to desiccation and freezing (Fryer 1996), and passage through the guts of fish, birds and mammals (Proctor 1964, Proctor *et al.* 1967, Mellors 1975), which is also important for surviving the dry phase in temporary water bodies.

4. DETERMINING THE FATE

The phenomenon of ephippia floating at the water surface is probably more widespread than previously thought and it is doubtful that mere physical processes such as winter storms (Kerfoot *et al.* 2004) or acquiring positive buoyancy in the sediments could be responsible for mass floating of ephippia observed in water bodies of different morphology and trophy (M. Slusarczyk and B. Pietrzak – unpublished). The hydrophobic outer structure of the ephippium makes it adhere to the water surface and increases its probability of being trapped by the forces of surface tension. How an ephippium carried by a female dwelling in the pelagial zone gets to the surface has only been investigated recently.

We studied (M. Slusarczyk and B. Pietrzak – unpublished) the mechanism of emergence of *Daphnia* ephippia at the water surface. With the use of an experimental set and video recordings we revealed that *Daphnia* females brought ephippia to the surface despite the direct threat of ultraviolet radiation which has been demonstrated to make *Daphnia* stay deeper in the water column. The females approached the surface only at the time of moulting and on having contact with the water-air phase boundary they were easily trapped due to the hydrophobic character of the ephippium. They freed themselves after moulting leaving the moult and the ephippium at the surface. All newly formed ephippia were negatively buoyant, but they could become buoyant after deposition at the surface, due to air penetrating the ephippial structure.

While all ephippia found at the surface had been brought there by the mothers, not all females shed the ephippium at the interface. Some females did not approach the surface and moulted in the water column, giving rise to the following questions: What influences a *Daphnia* female to moult at the surface or not? Is this behaviour indeed an adaptive mechanism facilitating dispersal? Do genes or environmental cues determine it? How are the further life histories of the animals different?

5. CONCLUSIONS

Whether entering diapause in *Daphnia* will provide a mechanism for dispersal in time, through survival over long periods of time, or dispersal in space, through the ability to be transported across otherwise uninhabitable parts of the environment, as put by Hairston (1998), might depend on the place of the deposition of the ephippium. This seems to be determined by the mother's behaviour, although we do not exclude the involvement of additional morphological traits. Floating poses different risks and advantages to the ephippium than sinking directly to the bottom. Whether these are two alternative survival strategies, remains an open question. If they are, prevalence of one or the other, accordingly to the theory, should depend on spatial and temporal variability of the habitat.

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Hide, rest or die: a light-mediated diapause response in *Daphnia magna* to the threat of fish predation

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SUMMARY

1. In a laboratory batch culture experiment, a diapause response of *Daphnia magna* to a simulated threat of fish predation was tested at various light intensities, which under natural conditions determine potential vulnerability of *Daphnia* to visual planktivorous fish.
2. Under moderate light intensity (1.4 μmol m^{-2} s^{-1}) that allows effective predation by fish, the proportion of females producing dormant eggs was significantly higher than under dim light conditions (0.001 μmol m^{-2} s^{-1}) that are not favourable for visual detection of prey. Production of dormant eggs was not observed in complete darkness or in treatments missing fish kairomones, irrespective of tested light conditions.
3. The observed phenomenon is interpreted as a flexible response of prey to the conditional risk of predation assessed by *Daphnia* according to the presence of fish-derived cues on the one hand and the presence of dark refugia on the other. Irrespective of the presence of fish kairomones, *Daphnia* may not produce resting eggs as long as a safe, dark, bottom zone is accessible.

Keywords: chemical communication, ephippia, kairomone, predator avoidance, refuge

Introduction

The relatively homogenous environment of open water does not offer pelagic organisms many places to hide from visually orienting predators. As light attenuates with depth in the water column, affecting the visual detection abilities of aquatic animals, many pelagic organisms diminish predator encounter rates by descending to the relatively safe, dark, bottom zone before sunrise and only ascending to subsurface water to feed after sunset (for a review see De Meester *et al*., 1999). However, the effectiveness of this diurnal mechanism may be affected by various environmental factors. The presence of a safe aphotic zone during the daylight hours depends on water depth and on the rate of light attenuation in the water column, which is affected by the quantity of suspended matter and watercolour. Depending on water transparency, the depth at which the safe, dark, refugium zone may be reached during daylight hours may range from tens of centimetres in turbid waters to tens or hundreds of meters in clear lakes and marine environments (Dodson, 1990; Ringelberg, 1995). Moreover, the daylight conditions may change substantially during the course of the season because of temporal changes in water transparency and seasonal or weather-dependent changes in light intensity impinging the water surface. In early spring, because of low algae production, or in June because of high grazing pressure on algae by abundant large-sized herbivorous zooplankton, light can penetrate deep into the water column. In late spring, summer, and autumn, water transparency is typically strongly reduced by abundant phytoplankton in eutrophic lakes (Sommer *et al*., 1986). In shallow lakes, water transparency may be additionally affected by resuspension of sediments on windy days. The presence of a dark refuge may also be determined by water level, which may change substantially during the course of the season in shallow

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water basins or man-made lakes. Even when a dark refuge is present, access to this zone may be permanently or temporarily obstructed by hostile conditions, such as the oxygen depletion which regularly occurs in the profundal zone of lakes of higher trophic status in winter or summer stratification periods (Sommer *et al.*, 1986).

When the effectiveness of diel vertical migration is reduced, to survive periods of predation in shallow or clear waterbodies, planktonic organisms should apply alternative defence mechanisms such as production of dormant forms (Hairston, 1987). The production of dormant eggs to avoid fish predation has been shown in a single species of calanoid copepod (Hairston, 1987) and in the cladoceran *Daphnia* (Ślusarczyk, 1995). Dormant forms of planktonic species may be relatively safe if they are resistant to ingestion or digestion or when deposited in places which are not accessible to predators (e.g. in the periodically deoxygenated hypolimnion). Dormant ('ephippial') eggs of *Daphnia* are highly resistant to digestion (Mellors, 1975) and can be found in large quantities in bottom sediments where they can stay viable for years or decades (Caceres, 1998). Although diapause may allow persistence in periods of low survival probability, it may reduce fitness of individuals because of extension of generation time and temporal retardation of development and reproduction (Cohen, 1966). Therefore, diapause seems to be an ultimate defence utilised only in extreme cases when active defence mechanisms are ineffective or cannot be used.

According to recent findings, poor food conditions (Ślusarczyk, 2001) as well as low yet tolerable temperature (Ślusarczyk, unpublished results) may determine the decision of *D. magna* to produce dormant eggs under a risk of fish predation. This reaction was claimed to be adaptive, as both lower food levels and low temperature may diminish the relative benefit of production of active offspring compared with the formation of dormant eggs when at risk of fish predation. Light conditions may influence this balance in another way, by determining the level of predation risk at particular densities of visual predators.

The aim of the present research was to test whether the diapause response of large-bodied, planktonic *Daphnia magna* to fish predation risk may be altered by light conditions, mimicking the presence or absence of a safe dark refugium in aquatic environments. Several other environmental factors have already been recognised as triggers of *Daphnia* diapause, namely: a short photoperiod (Stross, 1987), low temperature (Mortimer, 1935), and low food concentration when associated with other signals of environmental hostility such as crowding (Banta, 1939; Kleiven, Larsson & Hobaek, 1992; Alekseev & Lampert, 2001).

Methods

A clone of *D. magna* from Binnensee (Schleswig Holstein, N. Germany), a shallow, eutrophic lake in which *D. magna* coexists with fish (Lampert, 1991), was utilised in the experiment. *Daphnia magna* does not coexist with fish in many lakes as its large size makes it extremely vulnerable to visually feeding predators. The clone used in the experiment has been shown to respond to chemical signals associated with fish predation by changing its behaviour (Loose, von Ellert & Dawidowicz, 1993; Pijanowska & Kowalczewski, 1997), life-history (Weider & Pijanowska, 1993) and the induction of dormant egg formation (Ślusarczyk, 1995; Pijanowska & Stolpe, 1996). *Daphnia magna* from Binnensee is the only species known so far to produce dormant propagules in response to the presence of predatory kairomones.

Two experiments were performed. The first experiment tested an effect of light conditions and presence/absence of fish-derived chemical cues on a decision of *Daphnia* to produce dormant eggs. Experimental females were exposed to fish kairomones or water without addition of fish cues under three different light conditions: permanent darkness, permanent light, and the 'summer' daylight cycle (16 L : 8 D). The summer daylight treatment was intended to simulate light conditions of environments inhabited by *Daphnia* and lacking a dark refuge, while the dark treatment was intended to simulate light conditions faced by vertically migrating *Daphnia* in deep or turbid lakes. When hiding from visually hunting fish, *Daphnia* may face dark conditions permanently. It commonly spends nights in the subsurface zone and daytime hours in a dark, deep refuge. The permanent light treatment was used in the experiment to control for potential consequences of a change of photoperiod experienced by animals in the dark treatment that were moved from the summer daylight cycle (faced as embryos during pre-experimental period) to permanent dark conditions. Near-permanent light conditions may be experienced by *Daphnia* in summer at higher latitudes. Light intensity was set at a moderate level (1.4 μmol m^{-2} s^{-1}) in the experimental boxes during daylight hours. Such a light intensity should allow visually hunting planktivorous to detect prey in natural conditions (Blaxter, 1970). The experiment was run two times and employed equal number of fish and fish-free jars. Each treatment had six replicates. Data from the two runs were pooled and analysed together.

The second experiment tested more specifically the effect of different light intensities on dormant egg production by *Daphnia* when exposed to fish kairomones at a summer photoperiod of 16 L : 8 D. Three light intensities were tested: 1.4 μmol m^{-2} s^{-1} (called moderate light), 0.001 μmol m^{-2} s^{-1} (called dim light) and 0.000 μmol m^{-2} s^{-1} (called dark). The first treatment mimicked light intensities that should allow effective predation by visually hunting planktivorous fish (Blaxter, 1970). The second treatment imitated the light intensity close to the lower threshold (0.02–0.0002 μmol m^{-2} s^{-1}) indicated by Blaxter (1970) for visual predation by most tested planktivorous fish. Such light conditions may be found at a few meters depth in murky water of eutrophic lakes or at tens of meters in more transparent oligotrophic ones during daylight hours (personal observation). In the last treatment, experimental animals stayed in complete darkness over the study period. During the experiment, *Daphnia* were exposed to fish-derived chemical cues. Five replicates of each treatment were used. Moreover single batch cultures with no addition of fish cues were utilised for reference at each of the light conditions but were not considered as treatments in the statistical analysis because of lack of replication.

Experimental animals were enclosed in 0.9 L glass jars placed in cardboard boxes of predetermined light transparency at 22 ± 1 °C. Each cardboard box enclosed six glass jars. A single light source, a 120 W incandescent bulb, was placed 1.5 m over the experimental boxes. The cardboard boxes were opaque on the sides but were covered by lids with windows, the transparency of which was modulated by leaving a given number of sheets of white photocopy paper over it. This resulted in different light intensity in the different boxes.

The experimental media were prepared from aged lake water aerated for at least 48 h to remove potential predatory cues originating from the lake. This method was found effective in fish kairomone elimination (Loose *et al.*, 1993). The aerated water of the fish treatments was exposed to a small cyprinid fish, sunbleak (*Leucaspius delineatus*), at a density of two fish per 20 L of water, for 24 h prior to use. The two fish were fed with 20 chironomid larvae in total after the experimental media were prepared and water in a fish aquarium refilled. As at least two chemical cues, fish kairomones and alarm substance originated from injured *Daphnia*, were found necessary to induce diapause in *D. magna* (Ślusarczyk, 1999), the fish water was 'enriched' with prefiltered (GFC Whatman filter) homogenate of 10 *D. magna* per 1 L of experimental medium. Prior to use, experimental media were supplied with algae (*Scenedesmus obliquus*) at a concentration of 0.7 mg C L^{-1}. According to a previous study (Ślusarczyk, 2001), these food conditions should favour ephippia production in *Daphnia* exposed to fish kairomones when reared in batch cultures. For the first 9 days of the experiment, the volume of water was increased gradually over time as experimental animals grew (from 11 mL per individual at the first day of the experiment to 30 mL per specimen on the ninth day of the experiment and afterwards) and their filtration rates increased. Experimental media were changed daily.

All experimental females were likely to be genetically identical as they came from a line of females reproducing parthenogenetically. Experimental females, randomly chosen from a group of synchronously hatched offspring derived from a single female, were placed in experimental beakers within 20 h of birth. Twenty-eight neonate females were used initially in each experimental beaker. The mother females of experimental animals were kept at favourable food conditions (>2 mg C L^{-1}), summer photoperiod (16 L : 8 D) and a light intensity higher than 1.4 μmol m^{-2} s^{-1} before the experiments were started.

The number of females producing ephippia (the protective structures enclosing dormant eggs) and the number of newborn offspring were recorded daily. Females having ephippia were removed without replacement from cultures daily when the media were changed. This procedure allowed for reliable calculations of the frequency of ephippial females in each experimental jar and prevented miscalculation because of the possible multiple production of dormant eggs by a single female. Animal densities were maintained constant throughout the reproductive period by adjusting the water volume (30 mL per female) when individuals were removed. Daily manipulations of experimental animals from dark and dim light treatments were performed under red light illumination which, according to Young (1974), is hardly visible to *Daphnia*. The first experiment was continued until all females released their fourth-brood offspring, while the second one was finished when every animal had released its fifth brood.

Nonparametric Kruskal–Wallis ANOVA for ranks with sequential Bonferroni correction (Rice, 1989) was used for pairwise comparison of the proportion of ephippia-producing daphnids in experimental treatments.

Results

The results of both experiments indicate that light conditions may modulate a decision of *D. magna* to produce resting eggs when exposed to chemical cues of fish predation.

The first experiment indicated that the tested light conditions were alone an insufficient cue to stimulate production of dormant eggs in experimental clone of *D. magna* in the fish-free environment. Ephippia were not observed in any treatment lacking fish kairomones irrespective of light conditions (Fig. 1). However, when combined with fish-derived cues, the applied light conditions modulated diapause response in experimental *Daphnia*. In the two treatments where experimental females faced fish kairomones and moderate light intensity, production of dormant eggs was recorded. Under summer photoperiod, ephippial production was observed in all experimental jars in 26 ± 12% (mean ± 1 SE) of experimental females, whereas under permanent light conditions dormant eggs were recorded in four of six experimental jars in 24 ± 15% (mean ± 1 SE) of experimental females (Fig. 1). This recorded difference in diapause response between the two treatments was statistically insignificant. Concurrently, no ephippia production was observed in any fish jars kept in permanent darkness (Fig. 1).

A higher proportion of ephippial females was recorded in the second experiment, which tested diapause response of *Daphnia* to different light intensities at summer photoperiod and presence of fish kairomones. Ephippial production was observed in all experimental cultures where *Daphnia* was exposed to fish-derived cues in the moderate light treatment, with 62.0 ± 7.8% (mean ± 1 SE) of females forming ephippia, on average (Fig. 2). Under dim light conditions in the presence of fish kairomones the average proportion of females producing ephippia (8.7 ± 1.6%, mean ± 1 SE) was significantly smaller (Kruskal–Wallis ANOVA for ranks $H = 6.9, P = 0.009$). Ephippial females still occurred in all replicates. The proportion of ephippial females in the dim light treatment differed significantly (Kruskal–Wallis ANOVA for ranks $H = 7.8, P = 0.005$) from that in the dark treatment, in which diapause was not observed during the study period. Similarly, dormant eggs were not observed in the jars without predatory cues in any of the tested light conditions.

Discussion

While summer diapause of aquatic invertebrates inhabiting temporary waterbodies is commonly regarded as the mechanism of survival of periods of

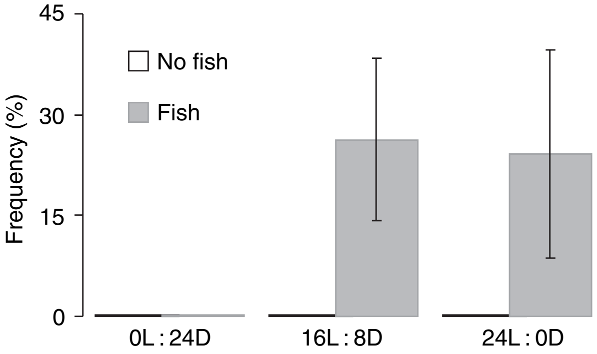

Fig. 1 The overall frequency (mean ± 1 SE) of ephippial females exposed to different light conditions (different photoperiod at moderate light intensity) in the presence or absence of fish kairomones.

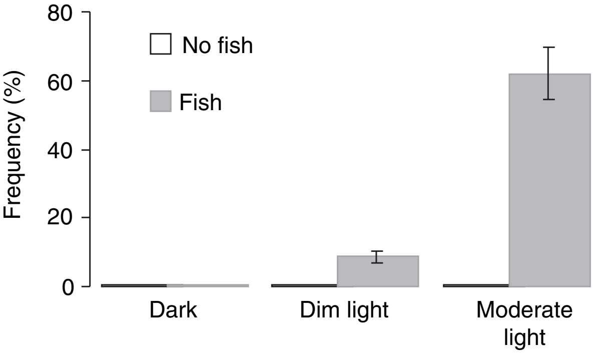

Fig. 2 The overall frequency of ephippial females (mean ± 1 SE) at the three different light intensities and summer photoperiod in environments with or without fish kairomones.

deterioration of abiotic conditions (Williams, 1998), it has less straightforward explanations in permanent lakes well buffered against abiotic changes. Summer diapause of planktonic crustaceans in permanent environments has been commonly attributed to two major biotic factors: deterioration of food resources because of intense intra- or interspecific competition (Banta, 1939; Santer & Lampert, 1995) or predation by fish or invertebrate predators (Threlkeld, 1979; Hairston, 1987). This seasonal phenomenon may be triggered by various environmental cues, which are directly (low food concentration, fish kairomones or metabolites of overcrowded population) or indirectly (specific photoperiod or water temperature) related to the ultimate forces. All these possible triggers may coincide during periods of summer diapause of the freshwater cladoceran *Daphnia*. This makes it difficult to judge the ultimate reason of this seasonal event under natural conditions (Threlkeld, 1979). Our experimental study extends the list of environmental factors that may affect the decision of *D. magna* to produce ephippial (dormant) eggs in summer.

The overall results of the two experiments indicate that light intensity may play an important role in the process of induction of dormant egg formation in *Daphnia* while under a threat of fish predation. As visually predating fish hunt more effectively in illuminated then in dim environments (Blaxter, 1970) the predation risk, and hence the need for prey defences, must be stronger under better light conditions. Chemical cues associated with fish predation may inform zooplankton on a potential danger of fish predation, which becomes regarded as a real threat by *Daphnia* when forced to stay in a bright environment. *Daphnia* may have a high likelihood of surviving in an active stage as long as a dark refuge is available. When the dark refuge is not present, or access to this zone obstructed by hostile conditions, the production of dormant eggs may become the ultimate defence assuring persistence of *Daphnia*. This modulation of the predator-induced responses by light intensity may be a more general phenomenon and could apply to other defence mechanisms too.

The presented results may explain the occurrence of multiple periods of dormant egg formation within a single summer, as observed by Lampert (1991) in the Binnensee, the lake from which the experimental clone was isolated. According to Lampert (1991) periods of dormant egg formation by *D. magna* coincided with a marked increase in water transparency and low food concentrations, likely caused by intense grazing of a dense *Daphnia* population. An increase in water transparency could have eliminated dark refugium zones in this shallow lake and hence could have exposed *Daphnia* to a higher risk of predation by fish. This agrees with the increased mortality at these times (Lampert, 1991). More intense predation by fish together with low food concentration could have diminished chances for *Daphnia* to survive and reproduce to below a threshold value where the production of dormant eggs becomes more beneficial than formation of active offspring (Ślusarczyk, 2001).

Although the experimental set-up did not allow us to test explicitly the occurrence of a trade-off between diel vertical migration and diapause in *Daphnia*, the presented results suggest this. The two mechanisms are likely triggered by the same ultimate force (danger of fish predation) and similar proximate factors (light intensity and presence of fish-derived chemical cues), which allow announcement of the danger in space and time. In the presence of visually hunting predators, we may expect prey to migrate downwards to a dark refugium. If for some reason (shallowness, high water transparency or oxygen depletion) a dark refuge is not accessible, the ultimate defence – production of dormant eggs – may be employed instead.

According to the results of our study, we may expect summer diapause of planktonic invertebrates to be favoured in shallow or transparent permanent lakes inhabited by fish, where the effectiveness of vertical migration is low. Factors like fish density, water transparency and temperature, as well as food conditions may fluctuate unpredictably in temperate lakes. This may explain why the mechanism of induction of summer diapause in *D. magna* may be modulated by so many environmental factors (Ślusarczyk, 2004).

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Fish faeces as the primary source of chemical cues inducing fish avoidance diapause in *Daphnia magna*

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Key words: kairomone, alarm substance, induced response, diapause, anti-predator defence

Abstract

In a laboratory batch culture experiment, females of *Daphnia magna* were exposed to five different experimental media containing either: (1) water from an aquarium with fish, (2) extract of fish faeces, (3) mixture of both media, (4) extract of homogenised conspecific *Daphnia*, or (5) control water without the addition of extra cues. The experiment was planned to test potential pathways of excretion of the chemical cues that induce resting-egg formation in *D. magna* and to find an effective way of collecting these chemical cues. The results indicate that fish faeces are the prevailing source of the chemical cues that induce resting-egg production in *D. magna*. The ease of collection and the possibility of storing it in a frozen state make it a convenient cue for inducing diapause response in *Daphnia*. The results of the experiment imply that in natural conditions *Daphnia* may face high concentration of the inductive signals once migrating to the bottom zone where fish faeces commonly accumulate.

Introduction

The production of resting stages, which are typically more resistant then active individuals to various unfavourable conditions, is a common way of genome protection in seasonally deteriorating environments. The diapause response is most widely associated with harsh abiotic (overfreezing, desiccation) and biotic (food depletion, overcrowding) factors (Danks, 1987). It seems to be also used by some freshwater crustaceans to avoid periods of high predation risk from fish (Threlkeld, 1979; Hairston, 1987). The mechanism of fish avoidance diapause may be triggered by indirect cues like photoperiod and temperature when the token cues are correlated with a seasonally occurring elevated risk of fish predation as indicated by studies on summer diapause of calanoid copepod *Diaptomus sanguineus* (Hairston & Kearns, 1995). It may be also cued by direct signals – fish kairomones, chemical compounds released into environment by fish predators as indicated by experimental studies on cladoceran *Daphnia magna* (Ślusarczyk, 1995; Pijanowska & Stolpe, 1996). Not only chemical composition but also the ways inductive substances are released into the environment remain unclear. At least three potential sources of inductive cues could be considered. They could be released directly from injured tissues of an attacked prey, excreted by fish through the surface of the body, or freed from fish faeces. Fish kairomones, which trigger behavioural defence reactions of *D. magna* were found so far in the fish faeces, mucus and intestinal tissues (Loose et al., 1993). Furthermore, the diapause response of *D. magna* to fish predation seems to be triggered by chemical cues of at least two origins: fish kairomones and some alarm substances coming from injured *Daphnia*. The diapause response of *D. magna* was observed when the two compounds were present in the environment simultaneously but not when in separation (Ślusarczyk, 1999).

The presented research was planned to test potential ways of excretion of chemical cues that induce formation of resting eggs in *D. magna* and to find out an effective way of collecting these cues. Determining the origin of the inductive cues will help in preparation of effective signals for diapause induction in follow-up experiments.

Methods

Cue collection

Thirty planktivorous fish (*Leucaspius delineatus*) with lengths between 5 and 7 cm were kept in 35-l aquarium for 24 h before they were fed with 3000 individuals of *D. magna*. All the fish were transferred by net to a small 5-l aquarium with 'fresh' water 1.5 h after being fed with *Daphnia*. This way potential cues, which might have been released from injured *Daphnia* during feeding of the fish, were not likely to be transferred to the new medium. For the next 6 h the fish were observed continuously in the small aquarium and all faeces was removed with a pipette once released by the fish. This way possible washout of the tested signals from the fish faeces was likely to be minimised. The collected faeces were stored temporarily in a refrigerator (4 °C) until the fish were removed from the small aquarium. Then the faeces were homogenised in 90 ml of water, centrifuged, filtered through a 0.45 μm filter, split into daily doses (i.e. 1.5 ml of extract per 5 l of final medium) between eppendorf vials and frozen at −25 °C shortly afterwards. Water from the small aquarium (henceforth called 'fish water') was filtered through a 0.45 μm filter, split into daily doses (80 ml of 'fish water' per 5 l of final medium) and frozen at −25 °C shortly after fish removal.

A further 300 specimens of *D. magna* were homogenised in 90 ml of water, filtered through 0.45 μm filter, split into daily doses (1.5 ml of extract per 5 l of final medium) and frozen at −25 °C.

The frozen 'fish water', extract of fish faeces and extract of homogenised *Daphnia* were used for the preparation of experimental media in the following experiment.

The experiment

A clone of *D. magna* originating from fish inhabited Binnensee lake in northern Germany known for its positive diapause response to 'fish cues' was tested in this experiment.
Experimental animals were exposed to five different media:

A – Control water without addition of the tested cues.
B – Control water mixed with frozen 'fish water' in a final concentration of 1fish per 10 l of medium.
C – Control water mixed with frozen extract of fish faeces. The final concentration of this mixture being the faeces from 1 fish per 10 l of medium.
D – Control water mixed with the two substances in matching concentration to B and C treatments.
E – Control water mixed with frozen extract of 10 homogenated *Daphnia* per 1 l of medium.

Each experimental treatment was performed in five replications except for the control, which was done in triplicate. The experimental media was supplied with 0.6 mg C/l of the unicellular algae *Scenedsmus obliquus*, used as a food source for *Daphnia* and was changed daily. The experiment was carried out in 0.9-l glass jars in 20 ± 2 °C and the summer photoperiod of 10D:14L. The small volume of medium (200 ml) in experimental beakers that was used initially was gradually increased to 900 ml by the seventh day as experimental animals grew and their filtering rate increased. Experimental females randomly chosen from a group of synchronously-hatched third generation offspring derived from a single female were placed in experimental beakers within 20 h of being born. Twenty-four neonate females were used initially in each experimental jar. All females had a high likelihood of being genetically identical since they came from a single line of females reproducing parthenogenetically. Newborn offspring were removed daily once the media was changed. Numbers of females producing ephippia (the protective structures enclosing resting eggs) were recorded daily. Females with ephippia were removed without replacement from cultures daily when the media was changed. This procedure allowed for reliable calculations of the frequency of ephippial females and prevented miscalculation due to the possible multiple production of resting eggs by a single female. Animal densities were maintained throughout the reproductive period as individuals were removed by adjusting the water volume (37 ml per female) in experimental beakers. The experiment concluded when all *Daphnia* had released five clutches of eggs. The frequency of females producing ephippia was used as a response variable.

Proportions of females producing ephippia were compared between treatments with *post-hoc* Tukey comparison made after one-way ANOVA test on arcsine normalised data.

Results

Various frequencies of ephippial females were observed in experimental treatments (ANOVA, $F_{4,18} = 72.2$, $p < 0.00001$). Almost all *Daphnia* produced ephippia when exposed to water containing extract of fish faeces and similar medium with addition of 'fish water' (Fig. 1). A potential additive effect of 'fish water' and fish faeces on induction of diapause in *Daphnia* could not be concluded due to the strong reaction of experimental females to the mere extract of fish faeces. A lower frequency of ephippial females (62.5% on average) was observed in the treatment containing 'fish water' only (Post-hoc Tukey HSD test, $p < 0.025$) (Fig. 1). The diapause response of experimental *Daphnia* was not observed in the control treatment and the medium with homogenised *Daphnia* (Fig. 1).

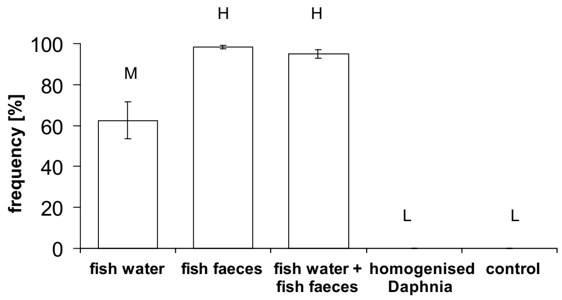

Figure 1. The frequencies of females producing ephippia (mean ± 1SE) in experimental treatments. Homogenous treatments are marked with the same letter: H – high values, M – medium values, L – low values (based on post-hoc Tukey HSD test, $p < 0.05$).

Discussion

The results of the experiment indicate fish faeces as being the main source of fish derived chemical cues inducing resting egg production in the tested clone of *Daphnia magna*. Unfortunately, the utilised method did not allow us to verify reliably whether inductive cues may be released through body surfaces of fish into surrounding water. It cannot be concluded that the positive reaction of experimental *Daphnia* to the 'fish water' observed in treatment B was caused by cues washed-out quickly from the fish faeces in the small aquarium during faeces collection and not chemical signals excreted by fish.

Results of the present experiment imply that *Daphnia* may face high concentrations of inductive cues in nature once fish faeces accumulate on the surface of bottom sediments. Specific feeding behaviour of *D. magna* (somersaulting above sediment surface) that causes resuspention of sediments eligible for filtration (personal observation) may expose it to a high concentration of fish kairomones. This could explain the relatively high threshold concentration of fish kairomones for defence reactions in *D. magna* observed elsewhere. According to Loose & Dawidowicz (1994), behavioural reactions of *D. magna* were marginal in a concentration of fish kairomones as high as 1 fish per 100 l of medium. Similarly, diapause response of *D. magna* was not observed when exposed to 'fish water' below a concentration of 1 fish per 50 l of medium (unpublished data). Both concentrations seem a few times higher than fish densities usually observed in natural conditions and could be understood in the light of the presented results.

The lack of diapause response to the extract of homogenised *Daphnia* supports previous findings (Ślusarczyk, 1999) indicating 'alarm substances' as insufficient cue for induction of resting egg production in the tested clone of *D. magna*.

The solid state of fish faeces made the preparation of a highly concentrated solution of chemical cues a relatively simply procedure. The ease of collection and storage in a frozen state makes it an experimentally convenient cue capable of inducing diapause response.

As long as the chemical composition of inductive cues remains unknown, a standardised method of cue preparation should be used to make possible the comparison of the results of different experiments on diapause induction. The presented procedure could be utilised as such. There are however, some potential limitations in utilising this method. The likely amount of inductive cues produced by a fish in a unit of time may be effected by the rate of fish defecation, which in turn is determined by the time of passage of gut contents, differs in different species and size of fish, and is commonly inversely related to the quantity of food eaten by fish (Hofer et al., 1982; Hofer & Nasir Uddin, 1985).

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Environmental plasticity of fish avoidance diapause response in *Daphnia magna*

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ABSTRACT

Organisms cope with harsh environmental conditions in various ways: either by tolerating environmental stress (through physiological adaptations), or by avoiding it in space (through migration) or time (diapause). Some species rely on a single strategy while others may choose from an array of options when facing different environmental stressors. Planktonic crustaceans may utilise different active (morphological, behavioural, life-history) or passive (diapause) defences to survive periods of high risk of fish predation. Recent evidence has indicated that resting egg production could be induced in Daphnia magna *by chemical cues associated with fish predation. This suggests that contrary to most known cases of diapause, which are triggered well in advance of catastrophic events (here termed "predictive diapause"), fish avoidance diapause in* D. magna *may exhibit a "responsive nature" and be initiated only after intensive predation appears. Experimental evidence discussed here indicates that the reaction of* D. magna *to chemical signals of fish predation could be conditional and determined by key environmental conditions, which in nature affect relative gains of activity vs dormancy. At high risk of fish predation, the decision of* Daphnia *to produce resting eggs was disfavoured by high food concentration. This reaction was claimed adaptive since high food allows for higher reproductive rates and better survival of offspring. All this may assure higher benefits due to activity despite some risk of predation (once predation pressure is not fatal to all active descendants) and disfavour resting eggs production. Moreover, at moderate food conditions the decision of* Daphnia *to produce resting eggs was disfavoured by the availability of a dark refuge from fish visual predators and thus likely lowering the risk of being preyed upon. Furthermore, when food was at a moderate level and a dark refuge was not present the decision of* Daphnia *to produce resting eggs was favoured by low water temperature. This could be explained as an adaptive reaction again, once low water temperature (due to its effect on a rate of metabolism) should have affected the gains derived from active life and reproduction more seriously than ones of inactive stages. The evidence presented here indicates that a responsive diapause allows* D. magna *to maximise reproductive output by taking advantage of opportunities presented by an unpredictable environment.*

Keywords: chemical communication, defence reaction, fish avoidance, conditional response, predictive vs *responsive diapause,* Daphnia

1. INTRODUCTION

Organisms cope with harsh environmental conditions in various ways: either by tolerating environmental stress (through physiological adaptations), or by avoiding it in space (through migration) or time (diapause). Some species rely on a fixed mode of defence, while others may choose from an array of options as they face different environmental stressors. Having a choice, organisms should employ that mechanism which maximises their reproductive success.

Most organisms cope with predation through various defensive responses. There are, however, environments that feature an exceptionally high risk of predation, e.g. open water habitats that do not offer many places to hide from visually orienting predators and expose whole populations of pelagic specimens to extermination (Gliwicz & Pijanowska 1988). There we can find organisms, which besides the widely used active anti-predatory defences produce resting stages to assure genome protection in periods of high risk of predation (Hairston 1987; Pijanowska & Stolpe 1996). Thanks to specific adaptations associated with slowed-down metabolism, dormant forms may tolerate a wider range of environmental conditions than active individuals, e.g. surviving passage through the digestive track of predators (Melllors 1975; Saint-Jean & Pagano 1995) or being deposited in places not accessible to predators and active individuals, e.g. the anoxic hypolimnion. Nevertheless, diapause would seem to be a costly defence, since, by entering it, individuals suspend propagation of their genotypes. Furthermore, although diapause may assure greater tolerance to harsh conditions, the survival of dormant forms may be far from complete for several reasons. The self-healing mechanisms that characterise active individuals are typically switched off or turned down in diapause. Thus, even the small injuries readily healing in active individuals may emerge as terminal in dormant specimens. Equally, as most resting stages are not able to control their position, their fate may be a matter of chance. They may be readily displaced into sites exposed to fatal conditions. Ultimately, successful survival of any hostile conditions must be rounded off by a resumption of development at the proper place and time. The perception of such favourable conditions may, however, be a challenge to dormant individuals when these are exposed to false signals, or else displaced into sites isolated from the cues that break dia-

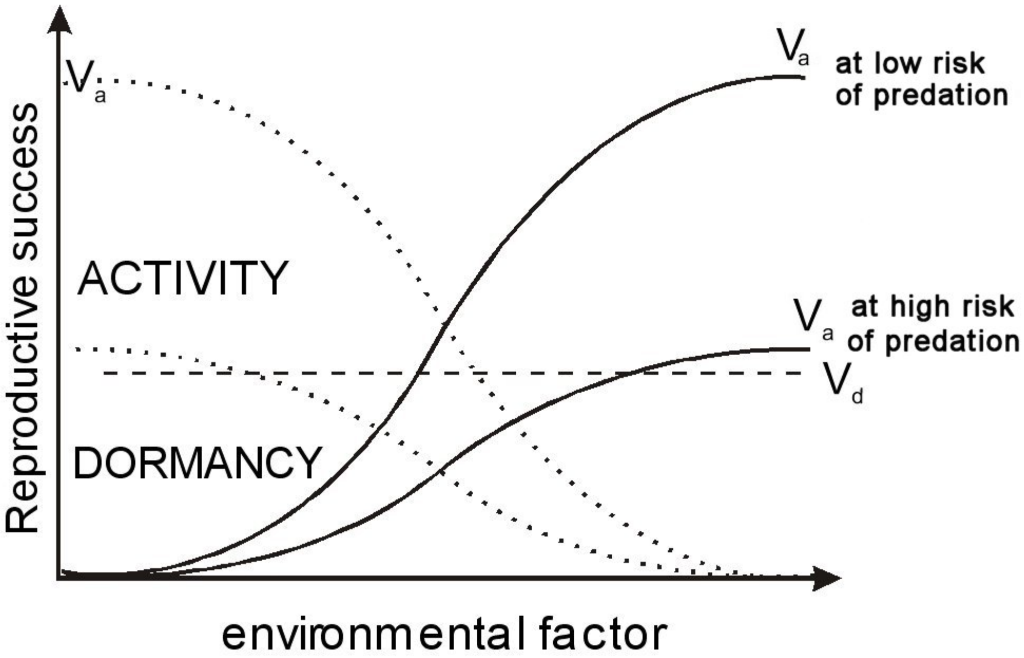

Fig. 1. Relative benefits of active life and reproduction (V_a) *vs* Diapause (V_d) at different levels of hypothetical environmental factor that affects chances for survival and reproduction of potential prey when exposed to different levels of predation (more details in the text.)

pause (Caceres & Tessier 2003). In consequence, dormant forms may thrive in diapause terminally or break dormancy spontaneously in an unfavourable place or season (Danks 1987). For all these reasons diapause seems to be a last-resort defence utilised when the protective mechanisms of active individuals prove ineffective.

1.1. Predictive vs *responsive diapause*

The mechanism underpinning the induction of fish-avoidance diapause may be initiated either in advance of, or after, the onset of a deterioration in the environment. The first mode will be termed here "predictive diapause", the second "responsive diapause". The diapause in the calanoid copepod E. sanguineus may be an example of the first kind. The species enters diapause well in advance of any intensification of predation pressure on the part of fish in a lake, in that the first individuals in a population enter diapause one generation or more beforehand (Hairston 1987), when informed of oncoming seasonal predation by a specific combination of factors involving photoperiod and temperature (Hairston & Dillon 1995).

The mechanism by which fish avoidance diapause is induced in the cladoceran *D. magna* could be used as an example of the other kind, i.e. of "responsive diapause". *D. magna* may produce resting eggs in response to a combination of fish kairomones and the alarm substances of injured conspecific prey (Ślusarczyk 1999), which appear in the environment when predation is already occurring. Both means by which diapause is induced have their flaws and advantages, and these will be discussed in a later section.

The large sizes of individuals of *D. magna* makes this species so extremely vulnerable to fish predation that it is typically found in small water bodies free of fish. However, thanks to a set of defensive responses, involving life-history (Weider & Pijanowska 1993), diapause (Ślusarczyk1995) and changes in behaviour (e.g., through diel vertical migration (Ellert & Dawidowicz 1993), swarming and enhanced vigilance (Pijanowska & Kowalczewski 1998)), *D. magna* has been successful in coping with fish in the shallow, coastal, eutrophic waters of the Grosse Binnensee in North Germany (Lampert 1989).

2. PLASTICITY OF DIAPAUSE INDUCTION

2.1. The hypothesis

The effectiveness of active defences, and hence the chances of *Daphnia* surviving and leaving viable offspring when facing predation, may be limited by various environmental conditions. If so, the limiting factors could potentially affect *Daphnia* decisions as regards the employment of the last-resort diapause response.

Let us consider a single hypothetical environmental factor which increases (solid line) or diminishes (dotted line) the chances for survival and reproduction of active individuals under predatory pressure in an asymptotic way (V_a lines) (Fig. 1) when it intensifies. It can affect the reproductive success of active individuals at various levels of predation (two levels of predation are actually shown on the graph). If we assume that the benefits of diapause (V_d) are small, and independent of wide ranges in the limiting factor (the rate of proliferation is low in diapause due to suspended development, while tolerance to various unfavourable conditions is commonly enhanced). For clearer presentation, a single V_d line is shown, and applies to both predation levels, though its value may in fact be somewhat affected by predation. Considering the given intensity of predation, there might be entirely different situations, depending on the level of the limiting factor. While individuals would gain more by continuing in active life and with reproduction at a high level (considering the solid lines on the graph), greater gains may derive from the formation of resting eggs when the level is low, i.e. when below V_d line. It seems that potential prey having a choice should apply that type of reproduction (active *vs* dormant eggs) which promises greater reproductive success. Predation level may shift the equilibrium point of benefits due to activity and dormancy to a different value of the limiting factor.

2.2. Testing the hypothesis

Here, I present briefly the experimental evidence to support the idea that the responsive diapause is flexible in nature, and go on to discuss its potential benefits. A detailed description of the studies invoked may be found elsewhere (Ślusarczyk 2001), or else will be published soon (Ślusarczyk *et al.*, unpublished manuscript; Ślusarczyk, unpublished manuscript).

Feeding conditions seem to represent the key environmental factor determining chances for survival and reproduction of individuals in the active state. At low food levels, a substantial decline is typically observed in growth and/or reproduction, and/or expenditure on defence. As a result, individuals grow more slowly and mature later, experiencing limitations on their chances of successful reproduction in a period of danger, giving rise to descendants that are few in number or of poor quality, and exposing themselves to a higher risk of predation where defences are not exhibited. Equally, when the food supply is not limited, the high reproductive effort of a well-fed, fast-growing prey individual displaying various defensive responses may ensure the survival of at least some descendants to reproduce - even when there is substantial risk of predation - and hence imply that the limited benefits of producing resting eggs are exceeded. To sum up, the production of resting eggs may offer greater benefits where feeding conditions are poor, only to give way to the more favourable production of active offspring at times when the food situation is better.

The probability of the production of ephippial eggs in *D. magna* exposed to a simulated threat of fish predation was determined in relation to food concentration in a batch culture experiment (Ślusarczyk 2001). In "fish-free" conditions, no production of resting eggs was observed with either low (0.4 mg C $l^{-1} \approx 20$ µg C ind^{-1}) or high (1.6 mg C $l^{-1} \approx 80$ µg C ind^{-1}) food availability, whereas in "fish" treatments, resting eggs were produced by various proportions of females in line with food concentration. With the limited food (20 µg C ind^{-1}) there was production of ephippial (dormant) eggs in the early broods of almost all females in the "fish" treatment. Where food concentrations were higher, proportions of ephippial females in the population were progressively lower, with females postponing the formation of resting eggs until later broods. At the highest food concentration (80µg C ind^{-1}), only about 17% of females produced ephippia when exposed to fish cues.

Water temperature resembles food in being able to affect both the quantity and quality of descendants. At low temperature (within tolerable range) ectothermic individuals typically produce larger offspring at rates a few-fold lower than when thermal conditions are optimal (Atkinson & Sibly 1997). Water temperature should not affect the benefits of dormancy where the metabolism of dormant forms and survival seem temperature-independent over a broad range (Danks 1987). Reproductive success in individuals producing active offspring may thus be lower at low water temperature, but higher under optimal thermal conditions, than that in individuals producing resting eggs when facing a high risk of predation. We might thus expect low water temperature to favour production of dormant offspring. On the other hand, water temperature may determine the energetic demands of fish predators, and thus the predatory pressure imposed on prey, which could affect the reproductive success of active individuals in the opposite direction. The overall effect of water temperature on the decision of potential prey to produce resting eggs could thus be ambiguous. Experimental evidence has in fact revealed a disfavouring effect of water temperature on the frequency of resting-egg formation in *Daphnia* exposed to a simulated threat of fish predation (Ślusarczyk, unpublished manuscript). At "low" water temperature (18 °C), all experimental females produced resting eggs in the early broods when facing the risk of fish predation and experiencing moderately good feeding conditions (0.25 mg C l^{-1} in a flow-through system). With increasing temperature (22 °C and 26 °C), and hence faster reproduction, but matching food conditions, the proportions of females producing resting eggs were significantly lower (44% *vs* 7%, respectively). The production of resting eggs in environments barren of chemical cues of predation was either observed at a very low level or was not present at all.

The disfavouring effect of water temperature on the frequency of occurrence of ephippial females may indicate either that water temperature does not increase the risk of fish predation as fast as it promotes the rate of *Daphnia* reproduction, or else that the risk of predation is anticipated by *Daphnia* in line with a signal other than water temperature, e.g. in accordance with the concentration of chemical cues associated with fish predation (Reede 1995).

Chances of prey surviving in the face of visually-oriented fish predators may be affected by light conditions, since the effectiveness of such predation may be limited at low light intensity (Blaxter 1970). As light levels attenuate quickly in a water column, deep or non-transparent lakes may offer a dark refuge from visually-feeding fish. *Daphnia magna*, like many other pelagic organisms, seeks to reduce rates of encounters with fish engaging in visually-oriented predation by spending daylight hours in the relatively safe, dark bottom zone, if this is accessible (De Mester *et al.* 1999). Individuals ascend to the warm and food-abundant subsurface water after sunset to enjoy a higher food concentration and the likely accelerated metabolism. However, the effectiveness of this diurnal mechanism may be influenced by various environmental conditions. Transparency of water or water level may change in the course of a season, determining the presence or absence of a safe refuge from fish predators in aquatic environments. When a dark refuge is not present, *Daphnia* must utilise an alternative defence, e.g. diapause, to survive. Indeed, the presence of a dark refuge seems to affect the decision of potential prey as regards their producing resting eggs. In laboratory batch culture experiments, a diapause response to chemical cues of fish predation in *D. magna* was tested for at various light intensities which in natural conditions determine the potential vulnerability of *Daphnia* to visually-orienting planktivorous fish. At a moderate light intensity which allows for effective predation by fish (1.4 μmol m^{-2} s^{-1}), the proportion of females producing resting eggs was found to be significantly higher than in dim light conditions (of 0.001 μmol m^{-2} s^{-1}), which are not favourable to the visual detection of prey (62% *vs* 8.7% of experimental females respectively produced ephippia). Production of resting eggs was not observed in complete darkness, nor in jars missing chemical cues of fish predation, irrespective of the tested light conditions (Ślusarczyk *et al.*, unpublished manuscript).

So far, three key environmental factors have been tested in this way: food concentration, water temperature and light intensity. All three have been shown to affect the probability of resting eggs being produced by *Daphnia* under a simulated threat of fish predation, but equally appeared non-influential in environments free from cues regarding predation. The list of modifying factors may likely be extended to include a few other conditions (e.g., food quality) that affect differentially the reproductive success of potential prey producing active *vs* dormant offspring. Could they affect the mechanism of induction of the predictive diapause in a similar way?

3. ADVANTAGES AND DISADVANTAGES OF PREDICTIVE AND RESPONSIVE DIAPAUSE

Typically, the predictive diapause is utilised to avoid highly predictable seasonal catastrophes (winter, dry seasons, etc). It is often initiated well in advance of unfavourable conditions, thereby allowing most individuals to enter diapause in time. The optimal time for diapause initiation seems to be one generation time or little more prior to the mean period of cyclical occurrence of harsh conditions in a population of unsynchronised individuals with a short generation time (Taylor 1980; Hairston & Munns1984). The life cycles of species of longer lifespan are commonly attuned to predictable environmental change, such that they may break the one generation time constraint and initiate diapause shortly before the environment deteriorates. Photoperiod or temperature are commonly used to trigger this. Since both environmental cues shift cyclically along with seasonal changes, the values for these signals that may be selected for are those that precede the appearance of any seasonal catastrophe with whatever degree of advancement may be adaptive. However, indirect signals do not inform organisms of the state of the environment at the time, but rather of the typical situation confronted by ancestors at that time of the year in the past. As a result, diapause may be initiated unnecessarily when a catastrophe does not take place incidentally, or else may not be employed in time when lethal conditions occur earlier then usual. Once selected, the strength of response and optimal period of diapause initiation may be adjusted to a new pattern of environmental change in the course of selection primarily (Hairston & Dillon 1990). The role of direct cues (informing of the state of the environment at the given time) in the initiation of the predictive diapause remains ambiguous (Danks 1987). It seems intuitively likely that the more the decision on diapause initiation precedes the period of appearance of hostile conditions, the less reliable the information on (future) chances for surviving the critical period in terms of its offering direct cues, and hence the less likelihood of their being utilised in the mechanism of diapause induction.

Not all environments deteriorate in a cyclical manner. Different strategies of diapause control are utilised to cope with unpredictable changes in environmental conditions: an extra-long diapause (of duration greater than one environmental cycle) (Cohen 1966), spontaneous production or dormant forms regardless of environmental conditions (Alekseev 1990) or the "responsive diapause" as a third choice. The advantage of the third option is its low costs compared to the other two, since it offers the shortest period spent in dormancy. The responsive diapause is initiated when the effects of an unfavourable factor are in progress, triggered commonly by cues directly associated with a selective factor (e.g., alarm signals from injured conspecific prey). This direct response may be modified according to other information on the present state of the environment, as indicated above. Therefore responsive diapause may be employed exclusively when real danger appears. However, the responsive strategy also has some serious drawbacks. Mobilisation of dormant forms may take considerable time (on account of the nature of the morphological, physiological and developmental transformations required to enter dormancy), such that dormant forms induced by the onset of hostile conditions may not be ready in time if the environment deteriorates abruptly. This mechanism of diapause induction thus seems to be employed in the face of conditions that deteriorate gradually, by organisms of short generation times (capable of fast mobilisation of the dormant forms). Predation may be such a condition. Unlike some terminal abiotic factors which develop abruptly (e.g., frost, flood or fire), predation pressure may develop gradually and as a selective force that is not fatal to all individuals in a population. After all, visually-orienting planktivorous fish seem to prefer older, bigger, more conspicuous prey. Younger, smaller prey individuals are under less intense pressure and may thus have more time to produce resting eggs, before being finally eaten by fish. In consequence, we may expect expression of the responsive diapause in young individuals primarily.

The responsive diapause may be readily confused with quiescence, since both responses display similar symptoms (retarded development), and both are initiated after the environment has actually begun to deteriorate. The retarded development of organisms in quiescence is a consequence of malfunctioning physiology and commonly disappears once conditions improve. In diapause, however, the metabolic retardation is a consequence of an internal mechanism triggered by environmental signals, and may last longer than the unfavourable conditions do.

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