

Effects of nest substrate on egg deposition and incubation conditions in a natural population of fathead minnows (*Pimephales promelas*)

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Abstract: Fathead minnows (*Pimephales promelas* Rafinesque, 1820) occur commonly in small lakes across the prairie region of North America. Males establish nests, court females, and tend the eggs. In our study population in Minnesota, males choose between two distinct nest substrates that differ in exposure to abiotic conditions affecting egg development: the underside of pond-lily (genus *Nuphar* Sm.) pads or the underside of submerged sticks. We found that lily pad nests contained more eggs, more clutches of different ages, and eggs distributed over a larger area than nests under submerged sticks. The difference in egg incubation temperatures between lily pad nests and stick nests varied diurnally with air–water temperature gradients. Proximity to the surface allowed lily pad nests to be significantly warmer during the day but only slightly cooler at night than nests under submerged sticks, resulting in about 10 h shorter time to hatch. The availability of lily pads for nests may greatly alter selection on sexual behaviour by males in competing for limiting nest sites of high quality, and female opportunity to exercise choice of mate. Moreover, availability of lily pads changes the role of abiotic conditions. In warm weather, males that nest under lily pads may achieve greater reproductive success than males that nest under sticks.

Résumé : Les têtes-de-boule (*Pimephales promelas* Rafinesque, 1820) se retrouvent couramment dans de petits lacs dans toute la région des prairies en Amérique du Nord. Les mâles construisent le nid, font la cour aux femelles et s'occupent des œufs. Dans la population que nous étudions au Minnesota, les mâles choisissent entre deux substrats de nidification qui se distinguent par leur exposition aux conditions abiotiques qui affectent le développement des œufs, soit la surface inférieure de feuilles de nénuphars (le genre *Nuphar* Sm.) et celle de bâtons submergés. Les nids sous les feuilles de nénuphars contiennent plus d'œufs, plus de masses d'œufs d'âges différents et des œufs répartis sur une plus grande surface que les nids sous les bâtons submergés. La différence de température d'incubation des œufs entre les nids sous les feuilles de nénuphars et ceux sous les bâtons varie pendant la journée en fonction des gradients thermiques air–eau. La proximité de la surface permet aux nids sous les feuilles de nénuphars d'être significativement plus chauds durant le jour, tout en étant seulement légèrement plus frais durant la nuit que les nids sous les bâtons submergés, ce qui réduit d'environ 10 h le temps d'incubation jusqu'à l'éclosion. La disponibilité de feuilles de nénuphars pour la nidification peut modifier grandement la sélection du comportement sexuel des mâles en compétition pour un nombre restreint de nids de haute qualité et les occasions qu'ont les femelles de faire un choix de partenaire. De plus, la disponibilité de feuilles de nénuphars change le rôle des conditions abiotiques. Dans des conditions climatiques chaudes, les mâles qui choisissent les feuilles de nénuphars peuvent peut-être obtenir un meilleur succès reproductif que les mâles qui établissent leur nid sous les bâtons.

[Traduit par la Rédaction]

Introduction

Environmental conditions during early embryonic development affect hatching success (reproductive success), time to hatch (extending period of exposure to predation risk and duration of parental investment required), and phenotype of hatchlings (antipredator competence). For example, water

loss from common starling (*Sturnus vulgaris* L., 1758) eggs create humid conditions in experimentally enlarged clutches that lead to a cooler nest microhabitat and lower hatch success (Reid et al. 2000). Black kites (*Milvus migrans* (Boddaert, 1783)) build their nests oriented eastward in response to prevailing winds and rainfall to maintain ideal incubation conditions (Viñuela and Sunyer 1992). In poikilotherms, incubation temperature has a strong effect on embryonic development, both in overall rate of development and in phenotype of the resulting hatchling (Qualls and Shine 1998). Exposure of embryonic tadpoles to physical disturbance (Warkentin 1995) or fish embryos to chemical cues of predators (Mirza et al. 2001) alters time of emergence and therefore phenotype at hatch, which in turn affects antipredator competence and likelihood of survival. The role of nest structure in regulating the temperature regime during embryonic development has received scant attention in fishes.

A critical interval in the lives of fishes is the period of

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early development because hatching success and survival of larvae determine reproductive success in fishes, and consequently, powerfully influence evolutionary trajectories (Miller et al. 1988; Divino and Tonn 2007). For fishes that guard their eggs, abiotic conditions (temperature, dissolved oxygen, water chemistry and salinity, water currents, etc.) of the nest site influence hatching success, which lead to the evolution of optimal nest-site selection.

The fathead minnow (*Pimephales promelas* Rafinesque, 1820) is a model organism for ecotoxicology and behavioural ecology. Surprisingly little is known about their natural reproductive behaviour in the field (Wynne-Edwards 1932; DeWitt 1993; Danylchuk and Tonn 2001, 2003, 2006; Bessert et al. 2007; Divino and Tonn 2007). Fathead minnows are small freshwater fish, typically 5–8 cm long, that live for 2–3 years in the wild. They are highly abundant in small lakes across central North America and often serve as a keystone species that regulate energy flow and productivity in prairie pothole lakes (Zimmer et al. 2001). These lakes are typically small, prone to winterkill by low dissolved oxygen, and colonized by only those few species tolerant of low dissolved oxygen (Klinger et al. 1982; Divino and Tonn 2007; Gale and Buynak 1982). Fathead minnows are polygynandrous, meaning that males and females may mate with more than one member of the opposite sex in a breeding season (Wynne-Edwards 1932; Markus 1934; Gale and Buynak 1982; Bessert et al. 2007). Males establish nests on the underside of horizontal objects such as rocks, pieces of wood, or vegetation (McMillan and Smith 1974). Only the male cares for the eggs in this species. Males invest heavily in reproductive behaviour. They fan the eggs to keep them oxygenated, rub the eggs with a specialized dorsal epidermal pad comprised mostly of mucus cells (Smith and Murphy 1974), defend the nest against egg predators and rival males, and court females (McMillan and Smith 1974; Unger 1983). During this time, males rely on somatic energy reserves for energy. When body energy reserves become low, males may consume some of the eggs in their care (Sargent 1989). Females can spawn up to 26 times during a breeding season, laying 400 eggs per bout for a seasonal (and usually lifetime) reproductive rate of 1000 – 10000 eggs (Andrews and Flickinger 1974; Gale and Buynak 1982). It is not known if females exhibit any fidelity to a male or nest site. However, it is known that females prefer to deposit eggs in nests that already contain eggs (Sargent 1988). Males sometimes engage in sneak fertilizations, or sometimes overt nest usurpations after which they tend any eggs sired by the previous male. Alloparental care increases the probability of new fertilizations for the alloparent (Unger and Sargent 1988; Bessert et al. 2007).

Nest-site selection can exert enormous influence on reproductive success because of its role in providing a nursery for early development and providing sanctuary from egg predators (e.g., Kvarnemo 1995). Nest-site selection by breeding males can take the form of water depth, region of the lake (DeWitt 1993), time of season (McKaye 1977), exposure to predators (Wisenden 1993), and incubation conditions within the nest (Jones and Reynolds 1999a, 1999b). In this study, we quantify egg deposition patterns and incubation conditions of fathead minnows that use two different nest substrates: the underside of the floating leaf of the pond-lily

(genus *Nuphar* Sm.; henceforth “lily pad” nests), or the underside of submerged tree branches (“stick” nests) (Fig. 1). These nest substrates differ markedly in exposure to atmospheric solar warming (and cooling) and to atmospheric oxygen, which should have significant consequences for conditions of egg development, and therefore to nest-site selection by males and mate choice by females.

Materials and methods

Study site

These data were collected in Budd Lake, Minnesota, within the boundary of Itasca State Park (ca. 5 ha; 47°09'59.35"N, 95°10'29.17"W). This lake contains only two species of fish; fathead minnows and northern redbelly dace (*Phoxinus eos* (Cope, 1861)). Population densities of both species are very high, creating conditions for limited availability of high-quality spawning sites. In June 2003, we collected 49 breeding male fathead minnows and the eggs in the nests that they were guarding: 25 nests were the underside of leaves of pond-lilies and 24 nests were on the underside of submerged sticks or bark. We used a backpack electroshocker to capture the male attending each nest and measured male total length to the nearest millimetre. We classified the eggs as either “eyed” (by the presence of dark pigmentation that develops in late stages of egg development just before hatching) or non-eyed (clear amber eggs that indicate early stages of development) (Fig. 1). Nests that contain eyed and non-eyed eggs almost certainly received eggs from more than one female (McMillan and Smith 1974) and potentially reflect a nest take-over (Unger and Sargent 1988; Bessert et al. 2007). We measured the length and width of the egg patch to calculate area. We measured the diameter of submerged sticks to the nearest millimetre with calipers.

For 30 days from 12 June to 10 July 2004, we monitored activity at 60 nests daily and recorded changes in egg numbers and hatching success. Nests were individually marked with flagging tape. Every day each nest was carefully lifted from the water and photographed with a high-resolution digital camera. Egg numbers and stage of development (eyed, non-eyed) were scored from the photographs using Image J version 1.34 (<http://rsb.info.nih.gov/ij/download.html>; accessed between September 2005 and March 2006). A maximum–minimum thermometer was checked daily. On the 20th, 27th, and 30th of June 2004, we used a YSI meter to record temperature and dissolved oxygen at the location of the eggs, and at 10 cm intervals of depth from the surface to the substratum. We took these measurements at three times during the day (0900, 1200, and 1500) for each of 15 nests for each nest substrate type.

Additional temperature data were collected from 29 June to 10 July 2005, using a HOBO® data logger with four probes to simultaneously record the temperature to the nearest 0.001 °C every 10 min of (1) air just above the water surface, (2) water just below the surface, (3) the underside of a lily pad, and (4) the temperature beside a submerged stick at a depth of about 20 cm, approximately 10 cm from the substratum. Values are reported as means ± 1 SE.

Fig. 1. Egg clutches of fathead minnows (*Pimephales promelas*) on the underside of a leaf of a pond-lily (*Nuphar* sp.) (left image) and the underside of a submerged stick (right image). The presence of eggs at different stages of development is indicated on the lily pad nest.

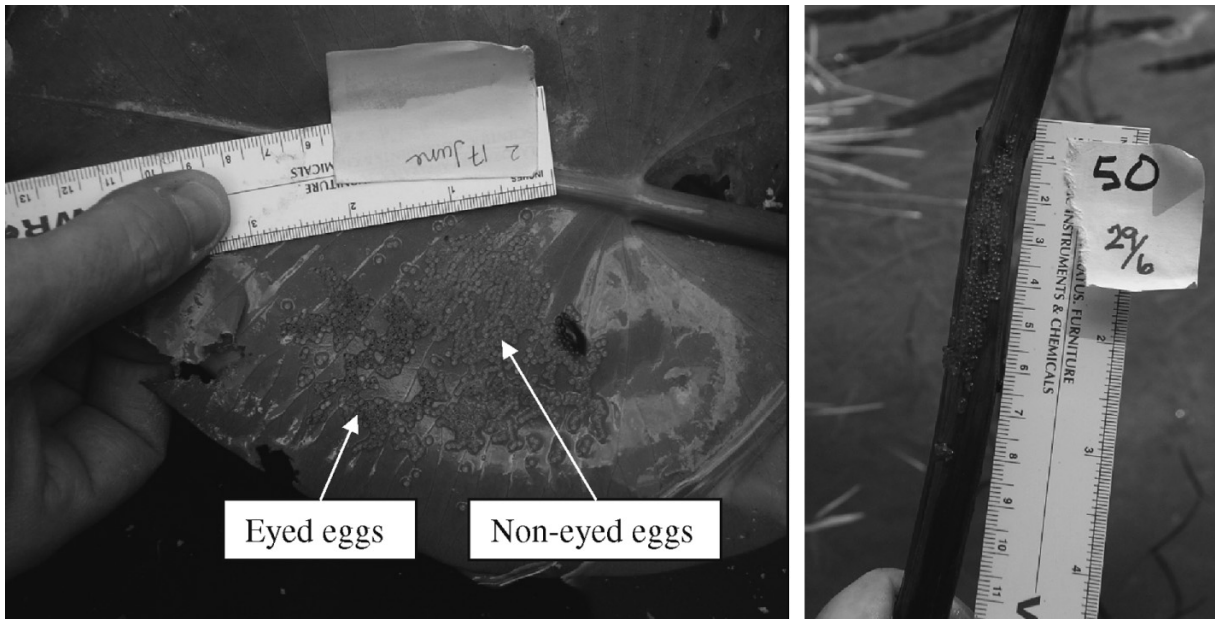


Fig. 2. Number of eggs in a sample of nests of different substrates, relative to the length of male fathead minnows (*Pimephales promelas*) guarding the nest. Open circles are nests on the underside of floating leaves of pond-lilies (*Nuphar* sp.), horizontal bars are nest on the underside of submerged sticks, and solid diamonds are nests under submerged pieces of tree bark.

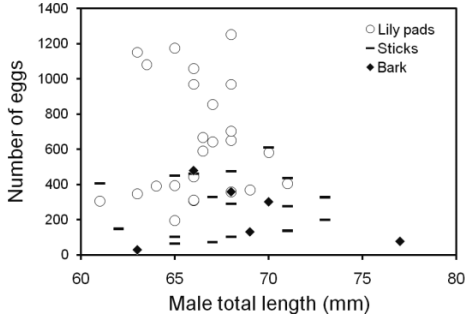
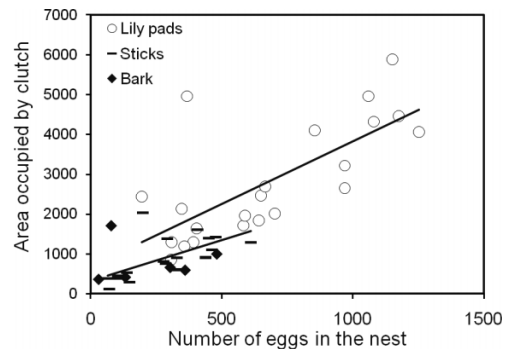


Fig. 3. Area (mm^2) of the nest substrate occupied by eggs of fathead minnows (*Pimephales promelas*) as a function of the number of eggs in the nest for nests under leaves of pond-lilies (*Nuphar* sp.), under submerged sticks, and submerged tree bark.



Results

2003 Data: point samples of egg number, clutch area, and male size

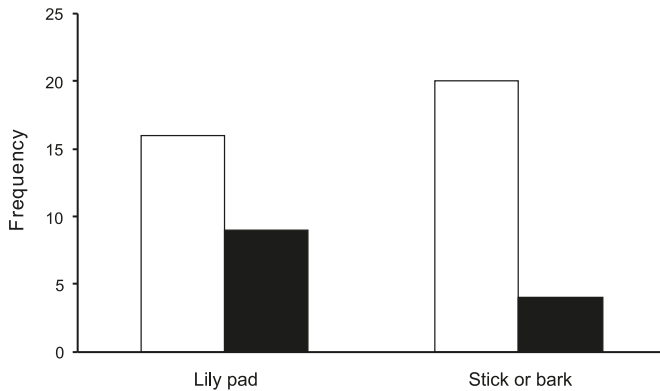
Nests under lily pads had significantly more eggs than nests under submerged sticks or submerged bark (Fig. 2). Male total length did not contribute significantly to variation in egg number (lily pad nests: 66.3 ± 0.5 mm, $n = 25$; submerged sticks: 67.5 ± 0.8 mm, $n = 19$; submerged bark: 68.8 ± 1.9 mm, $n = 6$); ANCOVA — nest type: $F_{[2,44]} = 10.83$, $P < 0.001$; male length: $F_{[1,44]} < 0.01$, $P = 0.990$; nest type \times male length: $F_{[1,44]} = 0.07$, $P = 0.930$). Lily pad nests had significantly more eggs (646 ± 65 eggs, $n = 25$) than nests on either submerged sticks (288 ± 38 eggs, $n = 18$) or bark (178 ± 73 eggs, $n = 6$) (Tukey’s test, $P < 0.05$). The area covered by egg clutches was significantly greater in lily pad nests (2822 ± 308 mm^2) than for either nests under submerged sticks (911 ± 124 mm^2) or submerged bark (791 ± 205 mm^2) (ANOVA — $F_{[2,43]} = 18.71$, $P <$

0.001 ; Fig. 3). Post hoc pairwise comparison tests on clutch area showed lily pad nests $>$ stick nests = bark nests (Tukey’s test, $P < 0.05$). Clutches under lily pads were almost always deposited as a monolayer, whereas eggs under submerged sticks were frequently deposited as a bilayer. Consequently, clutch areas for nests with fewer than 667 eggs were significantly larger for lily pad nests than submerged nests ($t_{[35]} = 3.51$, $P < 0.001$; Fig. 3). There was no effect of male length on stick diameter ($F_{[1,21]} = 2.10$, $P = 0.162$). Lily pad nests were also significantly more likely to contain eggs of mixed ages ($\chi^2_{[1]} = 4.39$, $P < 0.05$; Fig. 4).

2004 Data: longitudinal data on nest success, temperature, and dissolved oxygen at nests

We monitored 54 active nests containing eggs, 18 on the underside of a floating leaf of a pond-lily and 36 under submerged sticks. Water depth to the substratum was 21.5 ± 1.1 cm ($n = 54$) and did not differ between lily pad nests (22.9 ± 1.8 cm, $n = 18$) and stick nests (20.8 ± 1.5 cm, $n =$

Fig. 4. Frequency with which nests of fathead minnows (*Pimephales promelas*) in two types of nest substrate contained clutch(es) of only one age (all non-eyed or all eyed; open bars) or of different ages (contained both eyed and non-eyed eggs; solid bars).



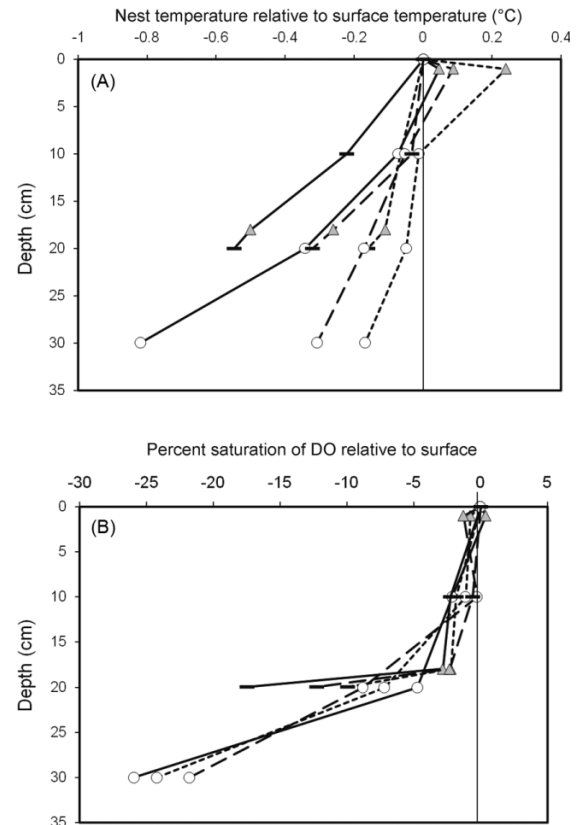
54) ($t_{[52]} = 0.87$, $P = 0.387$). Nest longevity, defined as the time period for which the nest continuously contained eggs, was 10.95 ± 1.9 days for lily pad nests but only 4.2 ± 0.6 days for submerged nests ($t_{[55]} = 4.06$, $P < 0.001$).

Temperature was higher under lily pads than submerged sticks by 0.35 °C at 0900, 0.55 °C at 1200, and 0.35 °C at 1500 (Fig. 5A). Eggs under lily pads were warmer than the water at the surface. Repeated-measures ANOVA showed a significant effect of nest type ($F_{[1,23]} = 15.99$, $P < 0.001$) and time of day ($F_{[2,46]} = 9.04$, $P < 0.001$), but no interaction between them (nest type \times time: $F_{[2,46]} = 1.45$, $P = 0.244$). Summer 2004 was unusually cold. Reproductive behaviour ceased completely on several occasions, particularly from 24 to 26 June when air temperatures dipped below 0 °C at night. Dissolved oxygen was significantly higher for lily pad nests than for stick nests, with no effect of time of day (repeated-measures ANOVA — nest type: $F_{[1,23]} = 12.05$, $P = 0.002$; time: $F_{[2,46]} = 0.20$, $P = 0.820$; nest type \times time: $F_{[2,46]} = 0.79$, $P = 0.458$; Fig. 5B).

2005 Data: continuous tracking of temperature using HOBO probes

We recorded temperatures every 10 min from 29 June to 10 July, for a total of 1728 observations at each of four locations ($N = 6912$). From these data, daily maxima and daily minima were calculated (Table 1). Overall, lily pad nests reached higher maximum temperatures during the day (paired t tests — $t_{[11]} = 4.53$, $P < 0.001$), lower minimum temperatures at night ($t_{[11]} = 3.19$, $P = 0.009$), and experienced a significantly wider range of temperatures ($t_{[11]} = 4.50$, $P = 0.001$) than nests under submerged sticks (Fig. 6). When air temperature was cooler than surface water temperature, water insulated egg temperatures from the cooling effects of the air, and lily pad nests and stick nests remained close in temperature (Figs. 7, 8). However, when air temperature (T_A) was warmer than water temperature (T_W), lily pad nests were warmed by up to 4.87 °C above the temperature found in submerged stick nests (Figs. 7, 8). Two relationships exist depending on which of air and water is warmest. When $T_A \leq T_W$, $T_{\text{lily}} - T_{\text{nest}} = 0.0957(T_A - T_W) + 0.4567$ ($R^2 = 0.095$, $F_{[1,1363]} = 143.41$, $P < 0.001$). When $T_A > T_W$, $T_{\text{lily}} - T_{\text{nest}} = 0.3432(T_A - T_W) + 0.3251$ ($R^2 = 0.357$,

Fig. 5. Temperature profiles (A) and dissolved oxygen profiles (B) for data collected in 2004 relative to surface temperature as a function of water depth, time of day, and nest substrate. Shaded triangles indicate the location of eggs of fathead minnows (*Pimephales promelas*) for each nest substrate type, and thus, temperature conditions for egg development. Nests under leaves of pond-lilies (*Nuphar* sp.) are denoted by open circles and nests under submerged sticks are denoted by horizontal bars. Short dashes indicate 0900, long dashes indicate 1500, and solid line indicates 1200 (noon).



$F_{[1,428]} = 237.51$, $P < 0.001$). The asymmetry between heating and cooling resulted in a thermal advantage for lily pad nests of about 3.9 °C (x intercept in Fig. 9). In other words, mean daily air temperatures 3.9 °C cooler than daily mean surface water temperatures produce the same mean thermal incubation conditions in lily pad nests and stick nests. The thermal advantage enjoyed by lily pad nests is derived mainly from residing in the surface film of water that warms quickly during the day, rather than by heat created through absorption of solar energy by the dark green pigments of leaves of pond-lilies (Figs. 5A, 6). Nocturnal cooling was ameliorated by the underlying layers of warmer water. The mechanism of this asymmetry is likely mediated by the effect of heat on water density.

When all 10 min temperature measurements were averaged over a 24 h period for the 12 days reported in Figs. 7 and 8, the incubation temperature, on average, was 23.74 ± 0.059 and 23.26 ± 0.051 °C ($n = 1728$ for each) for lily pad nests and stick nests, respectively. For fathead minnow eggs, time to hatch is a function of temperature (T): days = $26.04 - 0.88T$ (Andrews and Flickinger 1974). Using this equation, we estimate that the temperatures we measured would produce a time to hatch (days:hours:minutes) of

Table 1. Daily maximum and nightly minimum temperatures under leaves of pond-lilies (*Nuphar* sp.), on water surface, at stick nests, and in the air at Budd Lake from 29 June to 10 July 2005.

	Lily pad nest	Surface	Stick nest	Air
Daily maxima (°C)	26.19±0.75	26.00±0.70	24.90±0.62	30.60±1.50
Daily minima (°C)	21.84±0.56	21.94±0.55	21.76±0.67	17.24±0.78
Range (°C)	4.35±0.46		2.95±0.19	

Note: Values are means ± 1 SE.

Fig. 6. Temperature (°C) in nests of fathead minnows (*Pimephales promelas*) under leaves of pond-lilies (*Nuphar* sp.) (bold line) and under submerged sticks (thin dotted line) relative to the water surface temperature at Budd Lake in 2005.

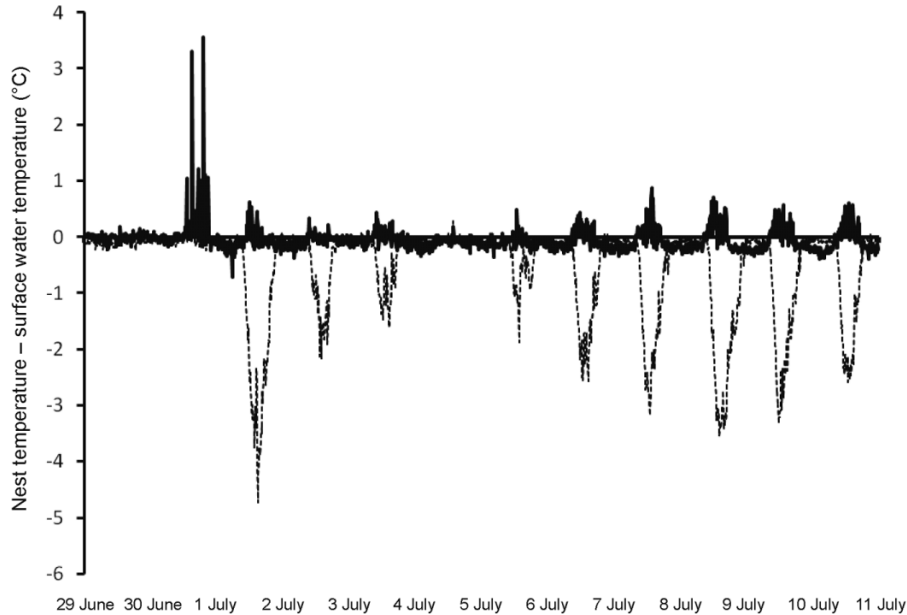
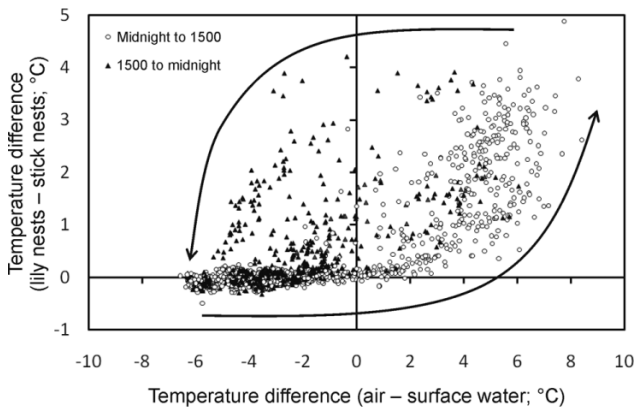


Fig. 7. Difference in temperature (°C) between nests of fathead minnows (*Pimephales promelas*) under leaves of pond-lilies (*Nuphar* sp.) and nests under sticks as a function of the difference in temperature between the air and the surface water from 29 June to 10 July 2005. Arrows indicate the temporal progression from early to late in each time interval.



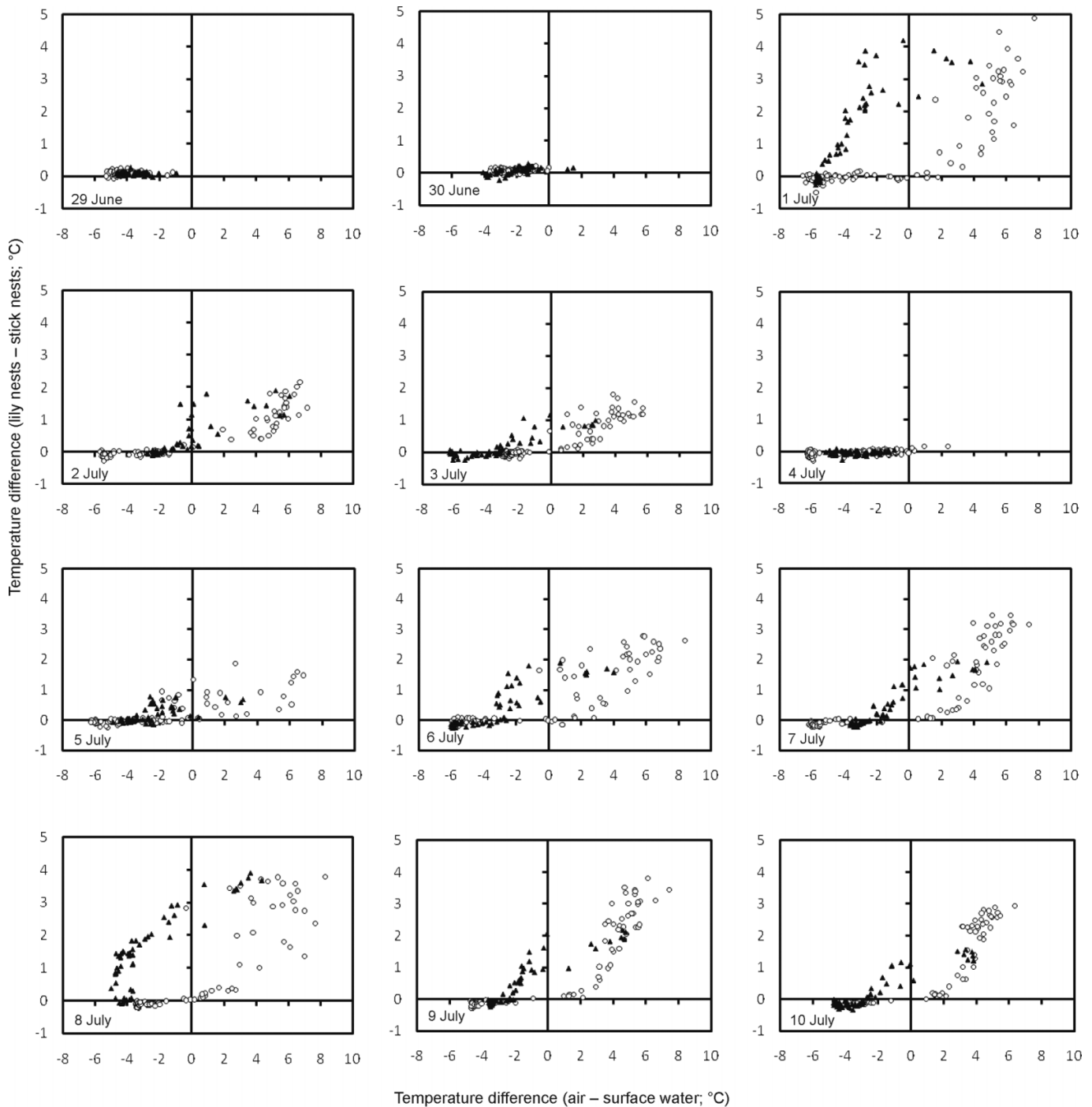
5:13:42 for eggs in stick nests and 5:03:34 for those in lily pad nests. Thus, incubation time in lily pad nests, on average, would have been about 10 h faster under the temperature conditions that we measured during the 12 day period presented in Figs. 7 and 8. Daily mean differences in tem-

perature between lily pad nests and stick nests track daily mean differences between air and surface water temperatures over the 12 day period (Figs. 7, 8). Concomitantly, temperature differences between nest types produce different hatch times (Fig. 9). Daily estimates of differences in hatch times between nest types ranged from -1 to +22 h (10 ± 2.25 h, $n = 12$).

Discussion

Choice of nest substrate has clear fitness consequences for reproductive success of fathead minnows. Relative to nests under submerged sticks, nests on the underside of lily pads contained more eggs, more clutches from different females, more clutches of different ages, enjoyed significantly longer duration of tenure as a functional nest, and fostered significantly warmer incubation temperatures and higher levels of dissolved oxygen. The asymmetry in heating and cooling is due to heating of the surface film in which leaves of pond-lilies reside. Budd Lake is sheltered from wind by steep embankments and riparian stands of mature red pine (*Pinus resinosa* Soland.) and eastern white pine (*Pinus strobus* L.). Under these conditions, thermal stratification occurs even in depths as shallow as 30 cm. Relatively low-density warmed water floats on the underlying layers of cooler water where submerged sticks are found. At night, cool night air cools

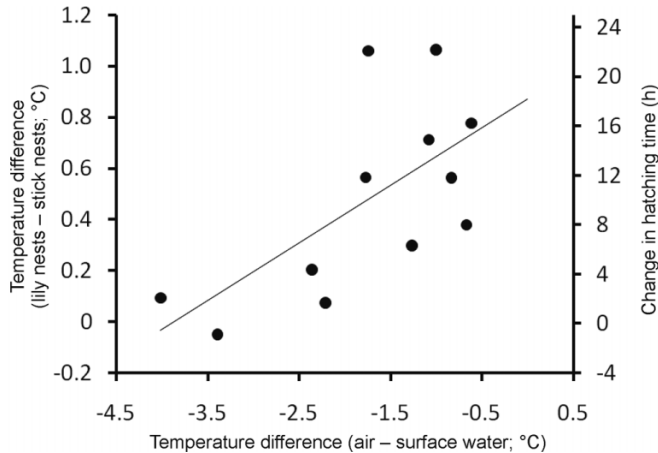
Fig. 8. Difference in temperature ($^{\circ}\text{C}$) between nests of fathead minnows (*Pimephales promelas*) under leaves of pond-lilies (*Nuphar* sp.) and nests under sticks as a function of the difference in temperature between the air and the surface water for individual days from 29 June to 10 July 2005. \circ , 2400 (midnight) to 1500; \blacktriangle , 1500 to 2400 (midnight).



the surface water film until the water density exceeds that of the underlying water, causing the cool water to sink. Thus, the surface water film, where leaves of pond-lilies are found, remains warmer than the night air because of the thermal inertia of the entire lake volume. In addition to the interplay of solar radiation and water density, there was a mild elevation of lily pad temperature above the temperature of the surface film during the day, perhaps owing to solar heating of the darkly pigmented leaves of pond-lilies.

All of these measures point to the prediction that lily pads are more successful and should be preferred to submerged sticks as a substrate for egg deposition. Counter-intuitively, size of males guarding lily pad nests did not differ from the size of males guarding submerged stick nests. This may reflect intense intrasexual competition for nest sites in this lake. A mark-recapture population estimate has never been attempted on Budd Lake, but anecdotally, it is clear that population density of fathead minnows is very high. For ex-

Fig. 9. Daily mean differences between nests of fathead minnows (*Pimephales promelas*) under leaves of pond-lilies (*Nuphar* sp.) and nests under sticks as a function of differences in daily mean differences between air and water surface. The temperature differential between nest substrates produces differences in predicted hatching time (h). Nest temperature differential ($^{\circ}\text{C}$) = $0.2205(\text{air} - \text{water temperature differential}; ^{\circ}\text{C}) + 0.8603$; time to hatch (h) = $4.6579(\text{air} - \text{water temperature differential}; ^{\circ}\text{C}) + 18.17$. For both regressions $R^2 = 0.4227$, $F_{[1,10]} = 7.32$, and $P = 0.022$.



ample, in June 2008, 60 standard minnow traps scented with sponges containing minnow skin extract (alarm cues that repel minnows from entering traps) at various stages of biodegradation captured a total of 4639 fathead minnows and 3390 redbelly dace (sum = 8029 fish) in 2 h at Budd Lake (Wisenden et al. 2009). With fish numbers of this density, male–male competition for nest sites is likely to be intense, resulting in only the largest males being able to secure a nest site (Danylchuk and Tonn 2001), and consequently, resulting in a narrow size range of breeding males for all nest substrates. Alternatively, the lack of a detectable difference in male size between nest types may reflect costs not measured in this study. Lily pad nests may be more difficult to defend from egg predators than stick nests are. A second possibility is that males guarding lily pad nests are more exposed to their own predators than males guarding stick nests (Candolin and Voigt 1998).

Submerged wood was not the preferred substrate of male fathead minnows in a population in South Dakota. In a comparison between two populations, Bessert et al. (2007) found nests under submerged wood occurred only in the population in which nest substrates were limited. They also found that male size was larger in the site with limited availability of nest sites. However, other factors may have differed between their two study sites that could affect size at reproduction (Smith et al. 1978; Danylchuk and Tonn 2006). In a study of a field population in Michigan, male fathead minnows preferred large tiles for nest substrates over small tiles, but no difference in male size was detected in males occupying these two types of nest (DeWitt 1993).

A family of beavers (*Castor canadensis* Kuhl, 1820) on Budd Lake contributed to the relative costs and benefits of nest types in two ways. First, through beaver foraging activity, there is a prodigious quantity of submerged sticks strewn about the littoral zone in Budd Lake. These are the

sticks used by fathead minnows for nest sites in Budd Lake. Availability of stick sites for minnow nests greatly exceeds the availability of lily pads as nest in our study site, although the majority of lily pad leaves remained unused by fathead minnows throughout our study. Secondly, beavers occasionally foraged on the floating leaves of pond-lilies (B. Wisenden, personal observation), representing a potential risk to selecting lily pads for a nest site. For unknown reasons, beavers were absent from Budd Lake in 2007 and 2008 (the time of this writing), which might change the costs and benefits of nest-site selection by fathead minnows.

Location on the water surface and exposure to direct sunlight permits lily pad nests to warm to a significantly higher temperature than nests under submerged sticks, which in turn leads to faster development, reduced exposure to egg predators, and potentially more brood cycles during a male's tenure on the nest. Chief among egg predators are shoals of northern redbelly dace that were observed on more than occasion to swarm a nest, overwhelm the defensive efforts of the guardian male, and consume all of the eggs. We also observed leeches (class Hirudinea) eating minnow eggs. We have no data on the relative exposure of the two nest substrate types to either of these sources of predation pressure.

Temperature affects development time of eggs generally (Pauly and Pullin 1988; Gillooly and Dodson 2000), and in fathead minnows specifically (Andrews and Flickinger 1974). Typically, the effect of temperature on embryonic development is measured and expressed as degree-days to time of hatch with temperature controlled at a constant value over the incubation period. Data from this study show clearly that this is not how eggs develop naturally. Our data concur generally with mean day and night temperature fluctuations reported by Markus (1934). Markus (1934) noted that water temperatures taken at 0800 and 1700 at nests of fathead minnows under submerged tiles differed, on average, by 4.1 ± 3.6 $^{\circ}\text{C}$ in June and July in Iowa. Future studies should explore the effect of temperature variation on the rate of embryonic development and the resulting larval phenotype.

Female mate choice, increased male fanning rate, and decreased egg development and male nest construction are all affected by low levels of dissolved oxygen (Jones and Reynolds 1999a, 1999b; Takegaki and Nakazono 1999; Lissaker et al. 2003). We detected only modest differences in dissolved oxygen levels in our study that would not likely be of sufficient magnitude to affect egg development or rates of egg fanning required of guardian males. Dissolved oxygen can be a challenge to fathead minnows over the long winter months of ice cover (Klinger et al. 1982; Danylchuk and Tonn 2001, 2006), but also at night in the summer when photosynthesis ceases and respiration of decomposers of organic materials in the substratum continue. If we had measured nocturnal dissolved oxygen levels, then we may have seen more pronounced differences between nest substrate types. This too is a potential avenue for future study.

The presence of eggs of different ages in nests could result from multiple spawns over time, or they could result from nest take-overs, whereby a male usurps the nest of another male and provides care for eggs sired by the previous male (Unger and Sargent 1988; Bessert et al. 2007). Nest take-overs occur more frequently when nest-site availability is limited (Bessert et al. 2007). In our study system, we pre-

dict that alloparental care is most likely to occur under lily pad nests because there is more area to accommodate multiple clutches and these nests have greater longevity. In this study, we did not determine the genetic parentage of the eggs or the longevity of males at individual nests. However, Bessert et al. (2007) found no correlation between nest substrate and parentage strategy despite differences in the numbers of eggs, numbers of eggs of different ages, and nest longevity.

This study reveals that reproductive success in fathead minnows is a complex interplay of biotic and abiotic factors which vary over time and space. Population densities of fathead minnows and northern redbelly dace, as well as the presence of beavers and lily pads, are all important biotic influences. Temperature and, to a lesser degree in Budd Lake, dissolved oxygen, interact with biotic factors to make nest-site-selection behaviour a dynamic agent of selection in the reproductive ecology of this model species. Recent attention on the potential impact of global climate change on prairie pothole ecosystems suggests that temperature-mediated effects deserve long-term monitoring. The fathead minnow seems well positioned to serve as a model species in yet another area of the biological sciences.

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