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Donald J. Padgett¹, Laura A. Horky¹ and Michiko Shimoda² : **Seed production and germination in endangered *Nuphar* (Nymphaeaceae) of western Japan**

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Abstract

Populations of both *Nuphar pumila* subsp. *oguraensis* and the partially fertile hybrid *N. ×saijoensis* are rare throughout their range. In an aim to identify factors that might influence conservation strategies, seed production and viability were evaluated in these two imperiled taxa and the more common *N. japonica* within the Saijō Basin, western Japan. Seed production was significantly higher per fruit in *N. japonica* and the lowest in *N. ×saijoensis*. Seed germination did not differ significantly between taxa, however the overall seed viability of the three Japanese taxa was low in comparison to other *Nuphar* taxa. Our findings suggest that seed viability is not a factor contributing greatly to the rarity of the *Nuphar* in the Saijō Basin. Fruit and seed harvesting techniques offer potential for propagation efforts.

Key words : germination, hybridization, *Nuphar*, Saijō Basin.

Introduction

Nuphar Sm. (Nymphaeaceae) is an aquatic genus of eight species and three known inter-specific hybrids (Padgett 1997; Padgett et al. 1998, 2002). The species are perennial and distributed primarily in north temperate freshwater ponds and streams.

Recent taxonomic studies of *Nuphar* by Padgett (1997, 1999) recognized two species in Japan : *N. pumila* (Timm) DC and the endemic *N. japonica* DC. The former species is represented in Japan by two subspecies : subsp. *pumila* of mostly northern areas and subsp. *oguraensis* (Miki) Padgett of central to southern areas. Other taxonomic interpretations of Japanese *Nuphar* (Ohwi 1965; Ohtaki and Ishidoya 1980; Kadono 1994) have recognized the latter taxon at the species level (i.e., *N. oguraensis*), in addition to recognizing a variant phenotype of *N. japonica* (i.e., *N. subintegerrima* (Casp.) Makino). Both *N. pumila* subsp. *pumila* and the endemic *N. pumila* subsp. *oguraensis* are rare in Japan and listed as threatened (Environmental Agency of Japan 2000).

The Saijō Basin (Hiroshima Prefecture) of

western Japan is an area ca. 10 km in diameter, east of Hiroshima City, distinguished by a high number (i.e., >1000) of artificial ponds. Most of these ponds are utilized for agricultural irrigation and serve as habitat for common and rare species of plants and animals (Shimoda 1985, 1993). Within the Saijō Basin, populations of *N. pumila* subsp. *oguraensis* exhibit an uncommon feature in having a distinctive red-colored stigmatic disk (The basis of Shimoda's [1991] *N. oguraensis* var. *akiensis*). These plants and those of *N. japonica* were found to naturally hybridize within Saijō Basin ponds (Padgett et al. 2002). The resulting partially fertile hybrids, *N. ×saijoensis* (Shimoda) Padgett and Shimoda, possess a distinguishable intermediate phenotype. Confined to only a limited number of ponds within the Saijō Basin, *N. ×saijoensis* (formerly *N. japonica* var. *saijoensis*) too is rare and listed as threatened (Hiroshima Prefecture 1995).

The present study expands our analyses of *Nuphar* in the Saijō Basin. Our focus was to specifically evaluate seed production and germination in the imperiled *N. ×saijoensis* and *N. pumila* subsp. *oguraensis*. Our aim was to iden-

tify factors that ultimately might influence propagation and thus aid *Nuphar* conservation and recovery strategies.

Materials and methods

Forty-four fresh fruits (21 of *N. pumila* subsp. *oguraensis* and 23 of *N. ×saijoensis*) were collected randomly from ponds within the Saijō Basin. In addition, fruits of *N. japonica* (12) were collected and utilized for comparison. Seeds were dissected out of ovaries and enumerated to calculate the mean number of seeds per fruit for each taxon. Means and standard deviations were calculated using the SYSTAT (version 5.0) software package (Wilkinson 1990). Means were compared between the taxa using a Kruskal-Wallis and Mann-Whitney U-tests.

In an attempt to exclude immature seeds, a subset of seeds from each taxon (776 seeds of *N. pumila*; 536 of *N. ×saijoensis*; 526 of *N. japonica*) was used for germination studies. The seeds of each fruit were stored in plastic Petri dishes filled with tap water and cold stratified (refrigerated for 5 weeks) to break dormancy

(Smits et al. 1990). To allow for germination, seed samples were exposed to a photoperiod of 15.5 hours under light banks (at room temperature). Light was provided by fluorescent “grow” lights to simulate natural light. Seeds were checked every two days for 35 d for germination (radicle emergence); distilled water was added to each dish when necessary to prevent desiccation. Mean germination was calculated and compared as above for each taxon. Cumulative percent germination was plotted against days of incubation to illustrate the rate of germination for each taxon.

Results

On average, fruits of *N. ×saijoensis* yielded the fewest number (29) of seeds (Table 1). Fruits harvested from *N. japonica* exhibited the greatest number of seeds (71 per fruit). Seed production ranged from 9–90 seeds per fruit among the two rare taxa (i.e., *N. ×saijoensis* and *N. pumila*), while some fruits of *N. japonica* yielded over 40% more seeds. Statistically, mean seed production differed significantly between *N.*

Table 1. Seed production in three *Nuphar* of western Japan

Taxon	n	Range	Mean seed no. per fruit (SD)
<i>N. ×saijoensis</i>	23	9–51	28.82 (10.12) ^b
<i>N. pumila</i> subsp. <i>oguraensis</i>	21	17–90	39.95 (20.34) ^{a, b}
<i>N. japonica</i>	12	26–130	71.25 (34.33) ^a

^{a, b} Means with different superscripts differ significantly ($p < 0.05$).

Table 2. Percent seed germination among *Nuphar* of western Japan

Taxon	N	n	Range	Mean (SD)
<i>N. ×saijoensis</i>	23	14	3–30	14.37 (9.14)
<i>N. pumila</i> subsp. <i>oguraensis</i>	21	11	2–67	22.14 (22.86)
<i>N. japonica</i>	12	8	5–38	15.73 (11.27)

Note: Means did not differ significantly ($p > 0.05$). Sample size N represents the number of fruits initially used in the germination study while sample size n represents the subset of fruits that ultimately yielded germinating seeds. Range and percent mean germination are based on n.

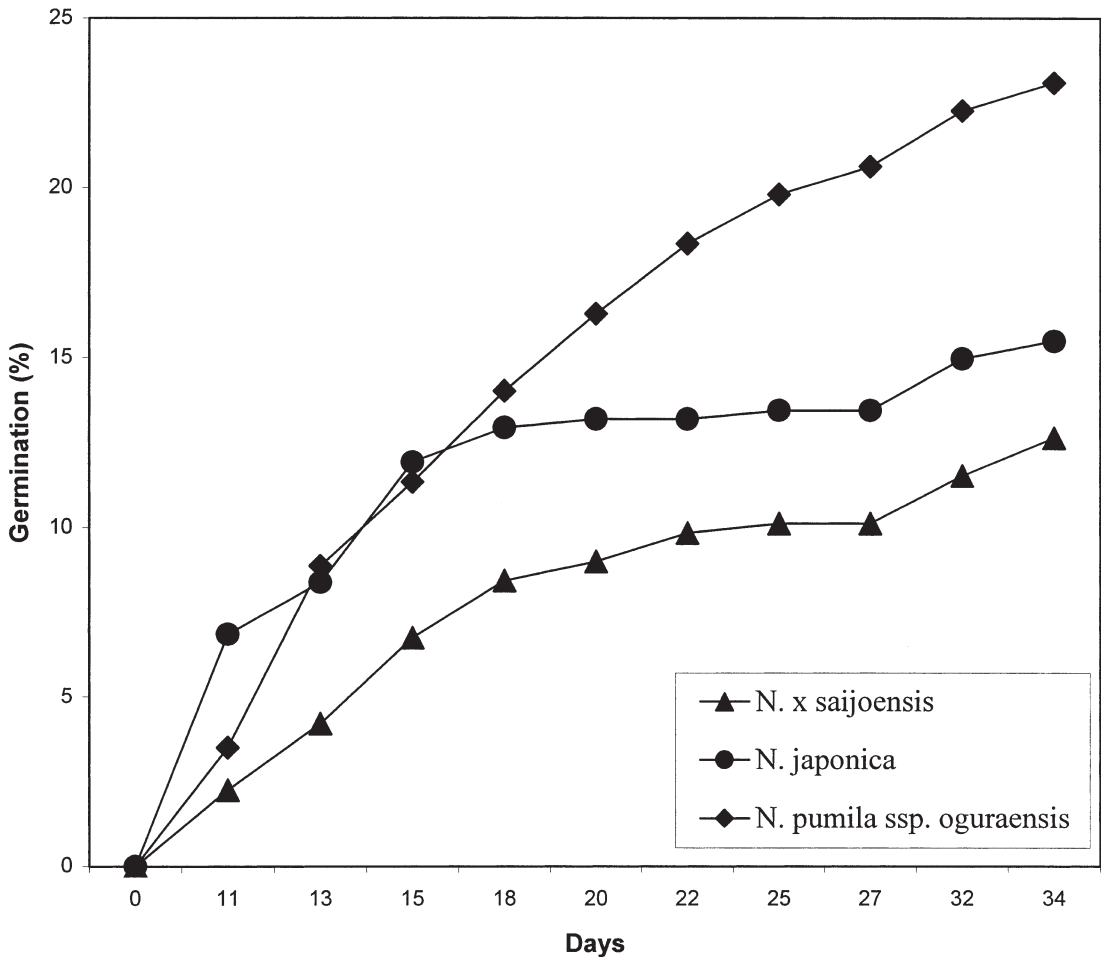


Fig. 1. The mean observed germination percentages of *Nuphar* seeds after cold stratification.

japonica and *N. x saijoensis* (Table 1).

Marked differences were apparent between the germinability of seed samples within each taxon. Not all of the fruits harvested from each taxon yielded viable seeds when allowed to germinate over a five weeks period. Eight of the 12 (67%) fruits sampled in *N. japonica* had at least one seed germinate. In *N. x saijoensis*, 14 of 23 (61%) samples had some measurable level of germination over five weeks, while 11 of the 21 (52%) samples of *N. pumila* germinated. Those samples that lacked germinating seeds were noticeably infected with a fungus or potentially immature. Of the germinating samples, *N. pumila* exhibited the highest mean seed viability (22% germination) and *N. x saijoensis* had the lowest (14%) (Table 2). However, there were no statistically significant differences between the mean

germination levels of the three taxa.

Under our experimental conditions, seed germination was initiated 11 days after light stimulation. The progression of the observed mean percentage of germination is given in Fig. 1. Daily sampling demonstrated the numbers of germinated seeds generally were highest during early incubation (i.e., first two weeks) and declined as time progressed (data not shown).

Discussion

Nuphar species so far examined are self-compatible yet dependent on insects for seed production (Ervik et al. 1995; Lippok and Renner 1997). Populations are able to produce large quantities of seeds capable of rapid dispersal (Hart and Cox 1995; Barrat-Segretain 1996). Vegetative reproduction via rhizome propagation

Table 3. Comparison of seed production among species of *Nuphar*

Taxon	n	Mean seeds per fruit (SD)	Range	Reference
Sect. <i>Astylus</i>				
<i>N. advena</i> subsp. <i>advena</i>	9	186.11 (58.50)	82-254	Smith et al. (1996)
<i>N. advena</i> subsp. <i>ozarkana</i>	6	41.00	43-72	Lippok et al. (2000)
<i>N. polysepala</i>	n/a	1416.00	n/a	Hart and Cox (1995)
<i>N. variegata</i>	10	266.40 (61.40)	146-345	Padgett, unpublished data
Sect. <i>Nuphar</i>				
<i>N. lutea</i>	15	285.00 (39.00)	232-348	Barrat-Segretain (1996)
<i>N. pumila</i> subsp. <i>pumila</i>	22	46.00 (39.00)	n/a	Lippok and Renner (1997)

n/a : data were not available.

is effective in dispersing and perpetuating populations but is reportedly slow (Smits and Wetzels 1986).

Several taxa of *Nuphar* are considered rare or declining within portions of their ranges (e.g., *N. microphylla*; Padgett 1998). Biological factors contributing to the rareness of such *Nuphar* are poorly understood, as are methods that might guide potential recovery strategies.

Much work has been done focussing on seed production and germination requirements in *Nuphar*. Average seed production per fruit varies markedly between species (Table 3) and can represent over 1,500 seeds m^{-2} in some populations (Van der Velde et al. 1978). In general, studies have shown that dry seeds lose viability quickly and a cold treatment is needed to alleviate dormancy (Smits et al. 1989, 1990, 1995; Smith et al. 1996).

Considering our data (of fruit from a single season), seed production among the Japanese taxa studied is generally low compared to most other species studied (Table 3), and interestingly lowest among the two rare taxa. The average seed output (40) of the rare *N. pumila* subsp. *oguraensis* is, however, comparable to that reported (46) for a conspecific subspecies in Europe (Table 3). Likewise, the low seed production evident in *N. ×saijoensis* is expected of an interspecific hybrid (Padgett et al. 2002). To speculate on the potential causes of the presumed low seed

production (i.e., resource or pollen/pollinator limitation) is beyond the scope of this paper.

Overall seed viability of the three Japanese taxa was low in comparison to studies of other *Nuphar* species (Table 2). For comparison, Smits et al. (1990) reported a mean germination of ca. 60% for the related *N. lutea* after a five-week incubation period (under similar conditions). Other studies have shown even higher mean germination figures (e.g., 93-100% for *N. lutea* [Barrat-Segretain 1996]; >80% for *N. advena* [Smith et al. 1996]). An overall low amount of seed fertility found among *N. ×saijoensis* samples is certainly expected as a reflection of its hybrid origin (Padgett et al. 2002). However, the lack of a significantly higher level of germination among samples of *N. japonica* and *N. pumila* remains puzzling, as does the steady decline in overall rate. Since our findings show low germination among the two threatened taxa as well as the common *N. japonica*, low germination rates do not appear to be a factor contributing greatly to their rarity.

In consideration of any potential recovery efforts, seed production levels in the imperiled *N. ×saijoensis* and *N. pumila* subsp. *oguraensis* appear suitable for propagation projects. Propagation and reintroduction of these rare aquatic plants and others (e.g., Suzuki et al. 1997) will help ensure their continuance while addressing the threatened taxa in Japan (Iwatsuki 1992).

Since it is impractical and destructive to collect *Nuphar* rhizomes (Smith et al. 1996), recovery efforts focussing on seeds would be reasonable and yield the highest level of genetic diversity. Seeds should be harvested from mature fruits however, since studies have shown that fruits fail to develop seed once detached from the stalk (Smith et al. 1996). Imposing anaerobic experimental conditions (Smits et al. 1990) and externally applied ethanol (Smits et al. 1995) could perhaps enhance seed germination levels of these imperiled taxa, but more study is needed. Additional study is also needed to quantify the levels of fruit production and seed-set among these rare *Nuphar*.

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- ドナルド パジエット¹・ローラ ホーキィ¹・下田 路子²: 西日本における絶滅のおそれのあるコウホネ属植物の種子生産と発芽
- 西条盆地(広島県)のため池には、以下の3種類のコウホネ属植物が生育している: コウホネ、ベニオグラコウホネ、サイジョウコウホネ。ベニオグラコウホネとサイジョウコウホネは稀少種であり、またサイジョウコウホネは不完全ながら稔性のある雑種である。これら3種類のコウホネ属植物の増殖に影響し、また保全・復元対策に役立つ可能性のある要因を検討するために、種子生産と発芽能力を評価した。果実1個あたりの種子数は、コウホネで有意に多く、またサイジョウコウホネにおいて最も少なかった。発芽率には3分類群間で有意な差は認められなかった。これら3種類の日本産コウホネ類の種子生産と発芽率は、他の地域のコウホネ属よりも低かった。これらの結果は、種子の発芽能力は西条盆地のコウホネ類の稀少性に寄与する主要な要因ではないことを示唆している。コウホネ類の種子による増殖は、地下茎の掘り取りよりも作業が容易で個体群への損傷が少なく、また遺伝的多様性を得られるため、稀少種の増殖対策において有効な手法と考えられる。
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