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ON THE NATURE OF THINGS: ESSAYS

New Ideas and Directions in Botany

Skimming the surface: duckweed as a model system in ecology and evolution

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With almost 400,000 species to choose from, plant biologists seeking a study species have no shortage of raw material. In the face of such diversity, plant model systems have come to the fore in many disciplines, with *Arabidopsis thaliana* being the most conspicuous example. The main benefits of model systems are that they (1) are amenable to the collection of data relevant to a variety of research questions (i.e., due to intrinsic species traits such as small stature and short generation time) and (2) come with value-added properties by virtue of their history of use in research, such as well-developed experimental protocols and molecular tools, a deep and broad literature, and an international community of experts (Fox, 2012; Chang et al., 2016). While the wisdom of relying on a small number of model systems is a matter of debate, there is little doubt that working on easily studied and well understood plant species has paid off in many disciplines.

Here we highlight the potential of one particular group—duckweeds—as a model system for research in the disciplines of ecology and evolution. Duckweeds are floating or submergent aquatic monocots that comprise the "simplest and smallest of flowering plants" (Hillman, 1961). Their small stature and morphological simplicity are just two of the many traits that make duckweeds well suited for addressing a variety of questions in ecology and evolution (Tables 1, 2). Furthermore, because of a long history of use in fields such as ecotoxicology (e.g., Wang, 1990) and plant development (e.g., Hillman, 1976), and ongoing interest in using duckweed for industrial applications such as feed and biofuel production (e.g., Cheng and Stomp, 2009) and bioremediation (e.g., Ziegler et al., 2016), researchers studying duckweed today benefit from a mature literature, established research procedures, repositories with hundreds of live strains, and ample molecular resources including genome sequences for *Spirodela polyrhiza* (Wang et al., 2014) and *Lemna minor* (Van Hoeck et al., 2015).

Before exploring the potential of duckweeds in ecology and evolution, we briefly review their natural history (based on Landolt, 1986). Individual duckweed ramets consist of a single "frond" (also called a thallus) with between zero and several roots emanating from the lower surface, depending on the species. Fronds are tiny, with surface areas on the order of 1 mm^2 to 1 cm^2. Though duckweeds can flower, the vast majority of their reproduction is asexual, via meristematic pockets from which clonal daughters successively bud and detach. An individual frond may produce up to a couple dozen daughters over its life, which is typically on the order of weeks. Taxonomically, the five genera and 37 species of duckweed form a monophyletic subfamily, Lemnoideae, within the family Araceae (though some authors prefer to put duckweeds into their own separate family, Lemnaceae; Sree et al., 2016). They are found in

TABLE 1. Example traits of duckweeds that make them suitable as model species in ecology and evolution. Additional notes and example references are provided.

Traits	Advantages
Intrinsic traits – Anatomy	
• Small stature (smallest angiosperms[1]) • Determinate growth (i.e., of ramets)[1] • Highly simplified body plan[1]	• Easy and inexpensive to collect, transport, store, and grow • Straightforward to cultivate in mesocosms, greenhouses, growth chambers, tissue culture chambers, and growth shelves • Can be grown in Petri dishes, facilitating axenic culture[2] • Facilitates large sample sizes
Intrinsic traits – Demography	
• Short lived (e.g., 18-39 days[3]) • Fast reproducing[4]	• Longitudinal and even multigenerational studies are feasible • Easy to build up large populations quickly • Amenable to experimental evolution (albeit with no recombination, typically)
Intrinsic traits – Reproduction	
• Mainly clonally reproducing[5]	• Ability to control for genetic variation • Don't need to worry about pollination or inbreeding (i.e., in lab populations)
Intrinsic traits – Diversity and distribution	
• Cosmopolitan distribution at the genus/subfamily level[5] • Diverse but tractable (37 species) • Often locally abundant[5] • Commonly co-occurs with ≥1 other duckweed spp.[5]	• Local access to populations for researchers around the world • Amenable to biogeographical studies and cross-species comparative work • Amenable to competition and coexistence studies • Ecologically important in many systems
Scientific community traits – Genetic tools	
• DNA barcodes available[6] • Draft genomes and transcriptomes available[7]	• Straightforward to identify cryptic species • Facilitates modern molecular ecology and evolutionary biology research
Scientific community traits – literature and community	
• Large literature[8] in biochemistry, ecotoxicology, genetics, industrial applications • Sporadic but long history in the ecology and evolution literature[9] • Large community of duckweed researchers, formal institutions[10] • Live specimens available from repositories[11]	• Continuity and synergy of research effort • Amenable to interdisciplinary studies and collaboration • Opportunities for replication

Table notes and references: (1) Hillman (1961); (2) Bowker et al. (1980); (3) *Lemna minor* CPCC 492 grown in modified Hoagland's E+ growth medium at 25 °C with a 12:12 photoperiod and a photosynthetic photon flux density of ~500 μmol m^{-2} s^{-1}; Barks and Laird (2015); (4) Ziegler et al. (2015); (5) Landolt (1986); (6) Borisjuk et al. (2015); (7) Wang et al. (2014); (8) see references within Landolt (1986) and a recent special issue of *Plant Biology* (January 2015, volume 17, issue s1); (9) See Table 2; (10) e.g., International Lemna Association, International Steering Committee on Duckweed Research and Applications; (11) e.g., Canadian Phycological Culture Centre (https://uwaterloo.ca/canadian-phycological-culture-centre/), Rutgers Duckweed Stock Cooperative (http://www.ruduckweed.org/).

TABLE 2. Example uses of duckweeds as a model system in ecology and evolution. Despite a long history of use in research, and the intrinsic and practical advantages of duckweeds (e.g., Table 1), the ecology and evolution research communities have never coalesced around duckweeds to the extent seen in other disciplines such as ecotoxicology and bioremediation, where they play a major role.

Topic	Example reference
Distributional ecology and island biogeography	Keddy, 1976
Population growth and population dynamics	Dickson, 1938
Population genetics	Vasseur et al., 1993
Demography and senescence	Ashby and Wangermann, 1949
Plant competition and coexistence	Vasseur et al., 1995
Alternative stable states	Scheffer et al., 2003
Plant-herbivore interactions	Van Der Heide et al., 2006
Non-herbivorous plant-insect interactions	Angerilli and Beirne, 1980
Plant-microbiome interactions	Gilbert et al., 2018
Evolution of adaptive plasticity	Vasseur and Aarssen, 1992
Bet hedging	Mejbel and Simons, 2018
Evolution on ecological time scales	S. P. Hart, M. M. Turcotte, and J. M. Levine, unpublished manuscript
Ecology and environmental science education	Robinson, 1988

lentic and slow-moving freshwater systems around the world and are particularly successful in systems subject to cultural eutrophication.

The duckweed traits that are most broadly useful in ecology and evolution are an extremely short lifespan and rapid rate of asexual growth. Duckweeds are ranked among the fastest growing and most productive higher plants (e.g., Ziegler et al., 2015), often completely blanketing the surface of their water body (Fig. 1). This rapid growth is part of what makes duckweed useful for industrial applications, but is also advantageous for fundamental research in ecology and evolution because it allows for a quick buildup of large sample sizes. Furthermore, the short lifespan of individual ramets makes it possible to track cohorts longitudinally and even multigenerationally, allowing in a matter of months the collection of data that would take years to obtain even in annual plants. For example, the relative ease of tracking cohorts over multiple generations has made duckweeds ideal for studying parental age effects in the contexts of both senescence (Ashby and Wangermann, 1949; Barks and Laird, 2015) and bet hedging (Mejbel and Simons, 2018).

Despite the dominance of asexual reproduction, populations of duckweed maintain relatively high levels of genetic diversity. For instance, Vasseur et al. (1993) sampled 157 unique genotypes of *L. minor* among eight small ponds in Ontario, Canada, separated by a maximum distance of just 12 km. In many parts of the world, at least a few duckweed species are locally abundant, with multiple duckweed species often co-occurring in distinct communities (Landolt, 1986). This diversity at multiple biological levels and spatial scales makes duckweed a good candidate for research on competition, coexistence, and the maintenance of biodiversity (e.g., Vasseur et al., 1995), as well as distributional ecology and biogeography (e.g., Keddy, 1976).

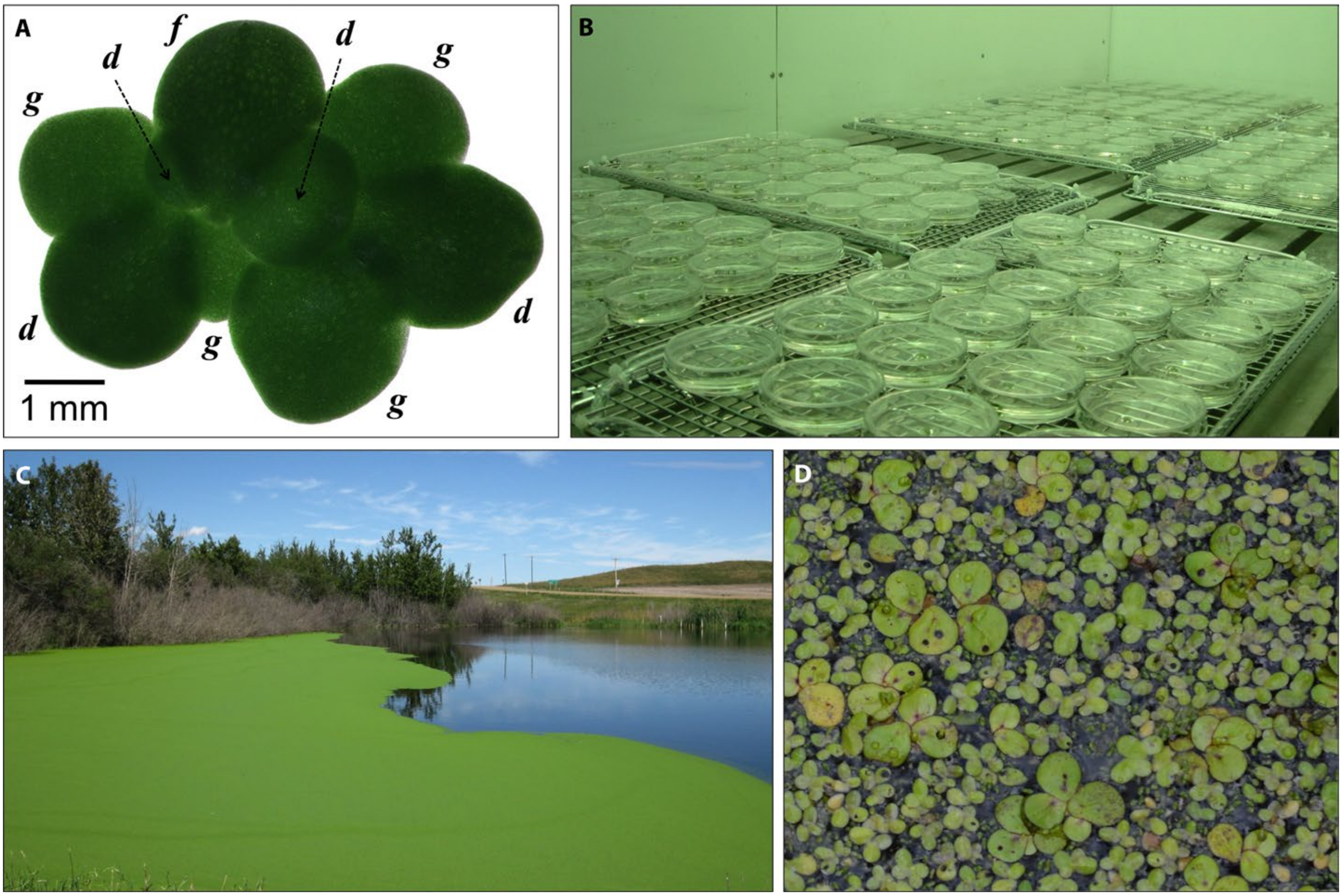

FIGURE 1. (A) A *Lemna minor* frond (*f*) with budding daughters (*d*) and granddaughters (*g*). (B) Growth chamber with Petri dishes housing individual *Lemna* fronds. (C) *Lemna* blanketing a prairie pothole wetland in south-central Alberta, Canada. (D) Mixed-species assemblage in northeastern Ohio, United States, composed of *Spirodela polyrhiza*, *Lemna minor*, *Wolffia brasiliensis*, and possibly *W. columbiana* (image and species information courtesy of Martin Turcotte).

Another trait that makes duckweeds amenable to research in ecology and evolution is the ability to tolerate a wide range of conditions, as might be supposed based on their widespread occurrence, and use as bioremediation agents in contaminated sites. One particular growth environment that can be useful for research in ecology and evolution is axenic culture (i.e., free of microorganisms), which allows for precise control over environmental conditions. Obtaining axenic cultures from wild-collected duckweed is straightforward (Bowker et al., 1980), and we have ourselves exploited this property to ensure constant environmental conditions in common garden experiments (Barks et al., 2018). Axenic culture is also useful in the study of plant microbiomes, a topic currently of intense interest (e.g., Gilbert et al., 2018). Finally, under axenic conditions, duckweed cultures can be maintained at low temperature for up to a year without requiring human intervention, which allows for simple long-term maintenance of stock cultures.

Of course, no model system is perfect for all questions, and some of duckweed's limitations must also be acknowledged. For instance, some duckweed species pairs are difficult to distinguish based on morphology alone; they can reliably be distinguished using laboratory manipulations or molecular identification techniques, but these approaches require time and money. In addition, because individual plants are small and typically free-floating, it can be challenging to mark and track individuals in a laboratory setting, let alone in the wild. That said, we think it will be possible to conduct individual-based studies in the wild using mark–recapture techniques or floating structures to limit plant movement—approaches we hope to pursue in the future. Furthermore, computer vision-based approaches are likely to become increasingly feasible for tracking and quantifying duckweed. Another potential limitation is that it may be difficult to generate de novo genetic diversity in duckweeds due to low rates of mutation and recombination (Xu et al., 2018 [preprint]); however, there have been successful efforts at flower induction and artificial cross pollination in some duckweeds (e.g., Fu et al., 2017). Finally, generalizing results based on duckweeds to other plant taxa must be done with caution, as some of the traits that make duckweeds easy to study (e.g., their size and anatomical simplicity; Table 1) also make them somewhat unusual. Relatedly, some of the functional traits on which plant ecologists commonly rely for comparative analyses are absent in duckweeds or at least difficult to compare with other plant taxa (e.g., height, rooting depth, phenology). These potential criticisms should be taken seriously when attempting to extrapolate results; however, we do not see them as a serious bar to using duckweeds in the first place.

Notwithstanding the long history of research on duckweeds in ecology and evolution (e.g., Table 2), their use in these disciplines has only "skimmed the surface" of possible applications. Indeed, despite what we argue are clear advantages to studying duckweeds, the ecology and evolution research communities have never coalesced around them to the extent of other fields such as ecotoxicology and bioremediation, where they continue to play a major role (Ziegler et al., 2016). Why not? Perhaps practitioners of ecology and evolution tend to favor theory and study species in which the mode of reproduction is primarily sexual; because duckweeds are almost exclusively clonal, they may not be a natural fit within the culture of these research communities. We also speculate that there may be more general resistance to model systems, particularly in ecology, compared to, say, molecular genetics or developmental biology (Fox, [2012] made a similar argument). Biodiversity is justifiably of perennial interest in ecology. Moreover, it is almost axiomatic that variation per se is often of direct focus in both ecology and evolution. It may be that these features predispose scientists in these disciplines away from model species, especially those like duckweeds whose charisma is subtle. Regardless, we think there are many fundamental questions in ecology and evolution for which duckweeds would be an ideal study system, particularly those whose study requires large sample sizes, short generation times, and easy experimental manipulability. To close with three representative examples, we foresee duckweeds as being highly profitable in studies on (1) experimental plant evolution, (2) processes that straddle the line between evolutionary and ecological timescales (an avenue beginning to be explored by S. P. Hart, M. M. Turcotte, and J. M. Levine [unpublished manuscript]), and (3) experimental tests of the effects of complex competitive networks (e.g., intransitive "rock–paper–scissors" competition) on strain or species coexistence.

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LITERATURE CITED

Angerilli, N. P., and B. P. Beirne. 1980. Influences of aquatic plants on colonization of artificial ponds by mosquitoes and their insect predators. *Canadian Entomologist* 112: 793–796.

Ashby, E., and E. Wangermann. 1949. Senescence and rejuvenation in *Lemna minor*. *Nature* 164: 187.

Barks, P. M., and R. A. Laird. 2015. Senescence in duckweed: age-related declines in survival, reproduction, and offspring quality. *Functional Ecology* 29: 540–548.

Barks, P. M., Z. W. Dempsey, T. M. Burg, and R. A. Laird. 2018. Among-strain consistency in the pace and shape of senescence in duckweed. *Journal of Ecology* 106: 2132–2145.

Borisjuk, N., P. Chu, R. Gutierrez, H. Zhang, K. Acosta, N. Friesen, K. S. Sree, et al. 2015. Assessment, validation and deployment strategy of a two-barcode protocol for facile genotyping of duckweed species. *Plant Biology* 17: 42–49.

Bowker, D. W., A. N. Duffield, and P. Denny. 1980. Methods for the isolation, sterilization and cultivation of Lemnaceae. *Freshwater Biology* 10: 385–388.

Chang, C., J. L. Bowman, and E. M. Meyerowitz. 2016. Field guide to plant model systems. *Cell* 167: 325–339.

Cheng, J. J., and A.-M. Stomp. 2009. Growing duckweed to recover nutrients from wastewaters and for production of fuel ethanol and animal feed. *Clean* 37: 17–26.

Dickson, H. 1938. The occurrence of long and short cycles in growth measurements of *Lemna minor*. *Annals of Botany* 2: 97–106.

Fox, J. 2012. Ecologists should quit making things hard for themselves and focus more on model systems. Blog post. Dynamic Ecology, https://dynamicecology.wordpress.com/2012/10/18/ecologists-should-quit-making-things-hard-for-themselves-and-focus-more-on-model-systems/ [accessed 24 May 2018].

Fu, L., M. Huang, B. Han, X. Sun, K. S. Sree, K.-J. Appenroth, and J. Zhang. 2017. Flower induction, microscope-aided cross-pollination, and seed production in the duckweed *Lemna gibba* with discovery of a male-sterile clone. *Scientific Reports* 7: 3047.

Gilbert, S., J. Xu, K. Acosta, A. Poulev, S. Lebeis, and E. Lam. 2018. Bacterial production of indole related compounds reveals their role in association between duckweeds and endophytes. *Frontiers in Chemistry* 6: 265.

Hillman, W. S. 1961. The Lemnaceae, or duckweeds: a review of the descriptive and experimental literature. *Botanical Review* 27: 221–287.

Hillman, W. S. 1976. Calibrating duckweeds: light, clocks, metabolism, flowering. *Science* 193: 453–458.

Keddy, P. A. 1976. Lakes as islands: The distributional ecology of two aquatic plants, *Lemna minor* L. and *Lemna trisulca* L. *Ecology* 57: 353–359.

Landolt, E. 1986. Biosystematic investigations in the family of duckweeds (*Lemnaceae*) (vol. 2). The family of *Lemnaceae* – a monographic study (vol. 1). Veröffentlichungen des Geobotanischen Institutes der ETH, Stiftung Rübel, Zürich, Switzerland.

Mejbel, H. S., and A. M. Simons. 2018. Aberrant clones: Birth order generates life history diversity in greater duckweed, *Spirodela polyrhiza*. *Ecology and Evolution* 8: 2021–2031.

Robinson, G. 1988. Experimental method and biological concepts demonstrated using duckweed. *School Science Review* 69: 505–508.

Scheffer, M., S. Szabó, A. Gragnani, E. H. van Nes, S. Rinaldi, N. Kautsky, J. Norberg, et al. 2003. Floating plant dominance as a stable state. *Proceedings of the National Academy of Sciences, USA* 100: 4040–4045.

Sree, K. S., M. Bog, and K.-J. Appenroth. 2016. Taxonomy of duckweeds (Lemnaceae), potential new crop plants. *Emirates Journal of Food and Agriculture* 28: 291–302.

Van Der Heide, T., R. M. M. Roijackers, E. T. H. M. Peeters, and E. Van Nes. 2006. Experiments with duckweed–moth systems suggest that global warming may reduce rather than promote herbivory. *Freshwater Biology* 51: 110–116.

Van Hoeck, A., N. Horemans, P. Monsieurs, H. X. Cao, H. Vandenhove, and R. Blust. 2015. The first draft genome of the aquatic model plant *Lemna minor* opens the route for future stress physiology research and biotechnological applications. *Biotechnology for Biofuels* 8: 188.

Vasseur, L., and L. W. Aarssen. 1992. Interpretation of adaptive plasticity in *Lemna minor*. *Oikos* 65: 233–241.

Vasseur, L., L. W. Aarssen, and T. Bennett. 1993. Allozymic variation in local apomictic populations of *Lemna minor* (Lemnaceae). *American Journal of Botany* 80: 974–979.

Vasseur, L., D. L. Irwin, and L. W. Aarssen. 1995. Size versus number of offspring as predictors of success under competition in *Lemna minor* (Lemnaceae). *Annales Botanici Fennici* 32: 169–178.

Wang, W. 1990. Literature review on duckweed toxicity testing. *Environmental Research* 52: 7–22.

Wang, W., G. Haberer, H. Gundlach, C. Gläßer, T. Nussbaumer, M. C. Luo, A. Lomsadze, et al. 2014. The *Spirodela polyrhiza* genome reveals insights into its neotenous reduction fast growth and aquatic lifestyle. *Nature Communications* 5: 3311.

Xu, S., J. Stapley, S. Gablenz, J. Boyer, K. J. Appenroth, S. K. Sree, J. Gershenzon, et al. 2018. Low genetic variation is associated with low mutation rate in the giant duckweed. *bioRxiv*, 381574 [preprint]. July 31, 2018 [cited 2018 Sept 9]. https://doi.org/10.1101/381574.

Ziegler, P., K. Adelmann, S. Zimmer, C. Schmidt, and K.-J. Appenroth. 2015. Relative *in vitro* growth rates of duckweeds (Lemnaceae)—the most rapidly growing higher plants. *Plant Biology* 17 (Supplement 1): 33–41.

Ziegler, P., K. S. Sree, and K.-J. Appenroth. 2016. Duckweeds for water remediation and toxicity testing. *Toxicological & Environmental Chemistry* 98: 1127–1154.