

1 **Benefits And Limitations Of Three-Dimensional Printing Technology For Ecological**
2 **Research**

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29 **Abstract**

30 **Background:** Ecological research often involves sampling and manipulating non-model
31 organisms that reside in heterogeneous environments. As such, ecologists often adapt techniques
32 and ideas from industry and other scientific fields to design and build equipment, tools, and
33 experimental contraptions custom-made for the ecological systems under study. Three-
34 dimensional (3D) printing provides a way to rapidly produce identical and novel objects that
35 could be used in ecological studies, yet ecologists have been slow to adopt this new technology.
36 Here, we provide ecologists with an introduction to 3D printing.

37 **Results:** First, we give an overview of the ecological research areas in which 3D printing is
38 predicted to be the most impactful and review current studies that have already used 3D printed
39 objects. We then outline a methodological workflow for integrating 3D printing into an
40 ecological research program and give a detailed example of a successful implementation of our
41 3D printing workflow for 3D printed models of the brown anole, *Anolis sagrei*, for a field
42 predation study. After testing two print media in the field, we show that the models printed from
43 the less expensive and more sustainable material (blend of 70% plastic and 30% recycled wood
44 fiber) were just as durable and had equal predator attack rates as the more expensive material
45 (100% virgin plastic).

46 **Conclusions:** Overall, 3D printing can provide time and cost savings to ecologists, and with
47 recent advances in less toxic, biodegradable, and recyclable print materials, ecologists can
48 choose to minimize social and environmental impacts associated with 3D printing. The main
49 hurdles for implementing 3D printing – availability of resources like printers, scanners, and
50 software, as well as reaching proficiency in using 3D image software – may be easier to
51 overcome at institutions with digital imaging centers run by knowledgeable staff. As with any

52 new technology, the benefits of 3D printing are specific to a particular project, and ecologists
53 must consider the investments of developing usable 3D materials for research versus other
54 methods of generating those materials.

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56 **Key Words:** 3D models, additive manufacturing, *Anolis sagrei*, clay model, Curaçao, Maya
57 Autodesk, sustainability

58 **Background**

59 Ecologists exhibit exceptional creativity and ingenuity in designing new tools and
60 equipment for their studies, often incorporating and repurposing technology from other fields.
61 For example, unique solutions have been devised for tracking animals (backpack-mounted radio
62 transmitters [1]), tracking seeds (fluorescent pigments [2]; seed tags [3]), catching animals (pit-
63 less pitfall traps [4]), containing or restraining difficult-to-hold specimens (squeeze box for
64 venomous snakes [5], ovagram for amphibian eggs [6]), and remotely collecting data or samples
65 (frog logger [7]; hair trap [8]), among countless others. Because many ecological studies require
66 customized equipment, ecologists are no strangers to building the contraptions necessary for
67 conducting their research, and the weeks leading up to and during field seasons and lab
68 experiments often involve multiple trips to hardware stores and craft shops.

69 Despite the high level of creativity and adaptability exhibited by ecologists, there is one
70 technology that ecologists have been slower to adopt relative to other fields: three-dimensional
71 (3D) printing. Additive layer manufacturing, or 3D printing, is the layering of material by a
72 computer-controlled machine tool to create an object from a digital file that defines its geometry
73 [9]. Most objects are printed in plastic, but newer print materials such as metal, wood, or other
74 composites are increasingly common in consumer applications. In the recent past (i.e., before
75 2010), 3D printing was cost-prohibitive and limited in availability, but it is now affordable and

76 accessible to budget-conscious ecologists. Many research institutions have at least one 3D
77 printing center and 3D printing services are available to all online. Other fields, such as the
78 health sciences, have readily adopted 3D printing into their research [e.g., 10], but it is as of yet
79 an untapped technology that ecologists can exploit to their advantage.

80 Recent studies have highlighted the benefits of 3D printing in terms of cost and time
81 efficiency [11,12], yet ecologists wanting to implement 3D printing for the first time must still
82 traverse a steep learning curve. Our goal here is to flatten the curve and provide ecologists with a
83 general but sufficient background in 3D printing technology to know what considerations are
84 important when approaching a 3D printing project. In this article, we provide an overview of
85 how 3D printing has been adopted by fields related to ecology. We highlight areas of ecological
86 research where we believe 3D printing has the promise to be most effective, and provide a
87 methodological workflow for integrating 3D printing into ecological studies. We illustrate this
88 workflow using an example from our own work, which includes the obstacles we encountered
89 and the solutions we devised. Finally, we conclude with important environmental sustainability
90 considerations.

91 *Overview of 3D printing in fields related to ecology*

92 Two disciplines that were early adopters of 3D printing technology and have strong
93 connections to ecology are biomechanics and natural history curation. Below we provide
94 examples of 3D printing implementations in these fields to provide ecologists with ideas of what
95 is possible.

96 The aim of biomechanics is to understand the movement and structure of living
97 organisms integrating across physics, engineering, physiology, and ecology. In biomechanics,
98 3D printing is used to test how the shapes of particular appendages or biological structures

99 function in the physical environment without having to use live organisms. For example, 3D
100 printed models of the sand-burrowing sandfish lizard's (*Scincus scincus*) respiratory system
101 made it possible to study why it does not inhale sand in ways that are impossible with an actual
102 respiratory system [13]. In studies of fluid dynamics, 3D printed models of swift (*Apus apus*)
103 wings and bodies of echolocating bat species permitted tests in water and wind tunnels
104 respectively to understand how morphology influences species' movements [14,15]. In other
105 applications, biomechanical theory is tested by attaching 3D printed structures to robots. In a
106 study of underwater burrowing mimetics in bivalves, Germann *et al.* [16] used mathematical
107 models to design a bivalve shell which was 3D printed and incorporated into a burrowing robot.
108 In other studies, evolutionary optimization models are used to design the shape of anatomical
109 structures. Then, 3D prints of the modeled and naturally occurring structures are compared in
110 performance tests to understand the evolutionary limitations species face in structural adaptation
111 [17,18]. For these studies, 3D models enabled scientific inquiry, as manipulating live animals
112 would have been challenging to impossible.

113 In the field of natural history curation, 3D printing increases the speed at which
114 discoveries are made, and the rate at which data and resources are shared across natural history
115 collections [19]. In paleontology, the reconstruction of complete skeletons is often impaired by
116 the recovery of incomplete remains at dig sites. Mitsopoulou *et al.* [20] used mathematical
117 allometric scaling models to calculate the dimensions of bones missing from the remains of a
118 dwarf elephant (*Paleoloxodon tiliensis*) recovered from Charkadio Cave on Tilos Island, Greece.
119 From these analyses, a 3D model was printed to allow the complete skeleton to be assembled. In
120 addition, 3D technology also facilitates the sharing of museum material without having to loan
121 valuable specimens, making it possible to construct complete skeletons using partial skeletons

122 from multiple separate collections [21]. In fact, museums have been quick to adopt 3D
123 technology because it vastly improves the rate at which collections are shared. The exchange of
124 3D-printed specimens facilitates crowd sourcing for specimen identification; access to high-
125 quality replicas of endangered, extinct, or otherwise valuable and/or fragile specimens; and
126 printed specimens can even be used in a field setting for species identification [21,22]. Museums
127 are increasingly accepting deposits of 3D printed material for rare and/or difficult to access
128 specimens. Lak *et al.* [23] employed 3D technology to describe two new damselfly species that
129 were preserved in amber. Because it is difficult to physically extract amber-encased specimens
130 without damaging them, the team used phase contrast X-ray synchrotron microradiography to
131 make 3D images of the specimens, and deposited the 3D prints in several museums. Finally, 3D
132 technology also accelerates the flow of information for education and outreach. For example,
133 Bokor *et al.* [24], developed a classroom exercise where students print fossilized horse teeth and
134 examine how the teeth changed over time with respect to changing climate.

135 *Integration of 3D printing in Ecology*

136 While ecologists have used 3D printing in a variety of applications (Table 1), there are
137 four areas where we view 3D printing to be the most impactful: behavioral ecology, thermal
138 ecology, building customized equipment, and enhancing collaboration.

139 The main goal of behavioral ecology is to understand how ecological and evolutionary
140 forces shape behavior. In addition to observational studies, behavioral ecology research can
141 involve manipulations of environmental conditions to test hypotheses. For testing hypotheses in
142 both lab and field conditions, 3D printing may be incredibly useful for making precise,
143 repeatable models. Three-dimensional printing has already been used to create precise models of
144 bird eggs to test egg rejection behavior in the context of brood parasitism [25], zebrafish shoals

145 to test the effect of body size on zebrafish shoaling preferences [26], and artificial flower corollas
146 to test the effect of floral traits on pollinator visitation [27–29] (Table 1). In these studies, 3D
147 printing was chosen for its ability to create identical experimental stimuli because alternative
148 methods, such as constructing models by hand, could introduce unintentional variation that
149 makes it difficult to determine whether study subjects are responding to intentional or
150 unintentional variation in experimental stimuli. In addition, 3D printing is often a faster method
151 for creating models than making them by alternative methods [12]. There may be scenarios
152 where 3D printing will not produce more biologically accurate models than other methods, but in
153 many cases, 3D printing will increase the types of behavioral questions that can be asked [25].
154 Within the field of behavioral ecology research, 3D printing can be used to test myriad behaviors
155 including predation (see Results), reproduction, foraging, social interactions, and defense in both
156 aquatic and terrestrial habitats.

157 Thermal ecology is focused on understanding how organisms are influenced by the
158 temperature profile of their environment. A major challenge of thermal ecology research is
159 constructing models that accurately replicate the thermal properties of a study organism. Copper
160 models are often used, however, recent work demonstrated that 3D printed plastic models were
161 cheaper and faster to construct and exhibited no difference in thermal properties compared to
162 standard copper models (Table 1) [12]. This, as well as the need for high numbers of identical
163 models, suggests that 3D printed models may make thermal ecology research more accessible.

164 Perhaps 3D printing will be the most helpful to the widest number of ecologists because
165 it provides a method for constructing customized equipment such as tools and experimental
166 habitats or mesocosms. In the field of soil ecology, 3D printing has been used to print artificial
167 soil structures which accurately replicate the macropore structure of soil (Table 1) [30,31]. These

168 artificial soils are ideal replicate experimental mesocosms for soil macro- and/or
169 microorganisms. Structures designed for other studies could be repurposed by ecologists as
170 experimental habitats such as artificial gravel beds originally designed for testing water flow
171 patterns [32] and artificial oyster shell reefs used to test how habitat complexity influences
172 predation rates [33].

173 Opportunities for printing tools are limited primarily by the ecologists' imagination and
174 range from simple structures to complex moving machines [34]. On the low-complexity end of
175 the spectrum, 3D printing has been used to sample two difficult-to-catch, invasive, tree-boring
176 beetle species that cause significant damage. Three-dimensional printed emergence traps make it
177 possible to effectively trap and census invasive ambrosia beetles (*Euwallacea fornicates*) as they
178 emerge from trees [35], while 3D printed decoys placed on standard beetle traps enhanced
179 capture rates of invasive emerald ash borer beetles (*Agilus planipennis*) [11]. In a more complex
180 application, whale researchers used 3D printing to build an unmanned surface vehicle named
181 *SnotBot* which allows scientists to get close enough to whales to collect biological samples
182 (Table 1) [36]. There are ample opportunities for ecologists to design tools to aid in data
183 collection, sample processing, organism containment, and even organization of field or lab
184 spaces.

185 From the examples provided above, designing custom materials certainly benefits
186 scientists within the context of a particular study. However, the use of 3D technology also
187 provides a mechanism for collaboration that extends beyond the limits of a single study.
188 Ecological studies that are replicated across systems, geographic boundaries, latitudinal
189 gradients, etc., are a powerful method for testing ecological theory [37]. The use of 3D
190 technology facilitates these broad-scale studies through the sharing of identical tools, models,

191 and/or equipment that can be used in multiple systems. For example, 3D printed models of
192 brown-headed cowbird (*Molothrus ater*) eggs [25] and Texas horned lizards (*Phrynosoma*
193 *cornutum*) [12] can be used to test patterns of brood parasitism and thermal tolerances,
194 respectively, across their geographic ranges. Similarly, for widespread invasive species like the
195 emerald ash borer, sharing effective trap methodology [11] among scientists and agencies can
196 potentially accelerate the rate at which the impact of the species is mitigated. In addition, 3D
197 technology provides a useful platform for ecologists who would like to incorporate citizen
198 scientists into a research program. Indeed, effective sampling technologies that can be
199 disseminated electronically are ideal for citizen science, and increase the speed at which
200 consistent data can be collected [38].

201 **Methods**

202 Below we describe a general workflow to use when embarking on incorporating 3D
203 printing into ecological research. Essentially, once an ecologist has identified the object to be
204 printed, the 3D printing process involves creating a printable 3D digital image file of the object,
205 selecting an appropriate print media, and then printing draft and final versions of the object
206 (Figure 1). To be clear, details specific to each project and available resources will need to be
207 explored and fine-tuned along the way. However, our workflow highlights the major steps and
208 aspects to consider at the onset.

209 *Make a digital object file*

210 The first step is to generate a digital file of the object to be printed, which can be
211 accomplished by creating a digital file of the image from scratch, converting a 2D image (e.g.,
212 photograph) into a 3D image, scanning an existing 3D object, or using an existing 3D file. All
213 digital 3D files require use of software specifically for editing 3D images (Additional File 1).

214 The most common 3D image file format is an STL file and is used by many software packages.
215 Depending on the image generating methods used and the types of modifications needed, there
216 may be a significant learning curve to attain the necessary level of proficiency on the software.
217 This is especially true for creating a 3D image completely from scratch (see below). In our
218 experience, however, we scanned an existing object and an undergraduate student was able to
219 work together with the printing center staff to learn the software and manipulate the image
220 within two months.

221 Before trying to create the image from scratch or scan an existing image, it may be
222 worthwhile first to check the many libraries of 3D imagery that are available online (Additional
223 File 2). It is possible that a digital 3D file of a similar object has already been created and can be
224 downloaded potentially for free, ready to be printed. Even if the file in an online library is not
225 exactly perfect, it can be manipulated using 3D software (Additional File 1), which, depending
226 on the modifications needed, may be a more efficient use of time than scanning an image or
227 trying to draft an image from scratch.

228 If a suitable digital 3D file is not available, but the object to be printed is in the
229 ecologist's possession, it is possible to use a 3D scanner to make a digital 3D image of the
230 object, similar to how a flatbed scanner makes a digital 2D image of an object. There are various
231 types of scanners, and it is necessary to choose a scanner that can accurately capture the level of
232 detail from the object being scanned needed for the project. Laser scanners, structured light
233 scanners, and even smart phone apps, can be used to create lower resolution scans of an object's
234 external features. Laser scanners were used to scan Texas horned lizards that were frozen in
235 realistic positions for a thermal ecology study (Makerbot Digitizer 3D, Makerbot, New York,
236 USA) [12], and oyster shells for a biomechanical predation study (Vivid 9i, Konika Minolta Inc.,

237 Tokyo, Japan) [33]. For more complex and fine scale objects with both internal and external
238 features like soil micropore structure, a method like X-ray microtomography is more appropriate
239 (HMX 225, Nikon Corp., Tokyo, Japan) [30].

240 If the object to be printed is not in the ecologist's possession, it is possible to design the
241 object using 3D drafting software (Additional File 1), with the time investment being
242 proportional to the researcher's proficiency on the software and the complexity of the object.
243 Using photogrammetry, photos can be digitized and 2D x,y coordinates from the photo converted
244 into a 3D image [25,39]. Photogrammetry may be the easiest and most cost effective method,
245 especially if a scanner is not available. Alternatively, mathematical formulae may be used to
246 generate different shapes, such as the surface of a bird egg [25] or the curvature of a flower
247 corolla [28]. Finally, it is possible to draft the object completely from scratch [e.g., 35], although
248 a higher proficiency on the appropriate drafting software is necessary (Additional File 2).

249 Once a digital 3D image file is in hand, it will likely need to be edited and customized for
250 the particular study. For example, in the brood parasitism study, the 3D image of the bird egg
251 was edited to make it hollow so that the printed versions could be filled with water so their
252 weight and thermal properties more closely matched a real bird egg [25]. Similarly, in the
253 thermal ecology study, the 3D image of the Texas horned lizard was edited to include a well in
254 the underside that fit a small environmental sensor (iButton) for measuring temperature [12].
255 Object size can also be manipulated and various polygons added to include additional structures.

256 Depending on the type of printer and material used, the image may need to be edited to
257 make printing possible and to efficiently use printing material. Non-manifold geometry errors
258 (i.e., geometry that cannot exist in the real world) can be common in scans made on biological
259 objects and must be corrected in order avoid fatal printing errors. Most 3D file manipulation

260 software allows for these corrections (Additional File 1). Because most printers print the object
261 from the bottom up layer-by-layer, any appendages or protrusions that extend out much wider
262 than the bottom layer may need added scaffolding to make the print possible. This scaffolding is
263 removed after printing is completed with varying degrees of effort depending on the design and
264 print material. In addition, if the object is not flat, it will likely need a flat base added to make it
265 printable. If multiple copies of the object are to be printed, it may be possible to rotate or stack
266 them so that several copies can be printed simultaneously. This method ensures efficient use of
267 printing platform space and materials.

268 *Printer and printing material*

269 There is a wide range of 3D printers that use various printing technologies and materials,
270 and a comprehensive review of all printer types is beyond the scope of this article. Here, we
271 focus on the printers and materials likely to be most useful to ecologists. Many factors must be
272 weighed when choosing a printer and printing material for a project, such as cost, material
273 durability, printed surface quality, timeframe for printing, and color. The most ubiquitous
274 printers that are common on university campuses and also through commercial online printing
275 services typically use either plastic-based filament or resin as the print material. Filament is hard
276 plastic stored on spools that is melted and deposited as beads or streams during printing that
277 quickly re-harden into layers to form the object. Resin is a polymer liquid that is layered and
278 solidified with UV light. Both come in a range of colors; filament is often cheaper but leads to a
279 lower resolution print with printed bands more prominent on the finished object, however if
280 needed there may be applicable surface finishing methods for smoothing out these bands, like
281 using acetone vapor. Filament may also be less durable for some applications and cracks can
282 form between layers if the object is subjected to physical stress. Finished resin products are

283 generally smoother, can be printed at higher resolution, are more durable, and have the surface
284 quality of a store-bought plastic item.

285 Both filament and resin have been used for printing low and high resolution ecological
286 models, respectively. For example, acrylonitrile butadiene styrene (ABS), a type of filament, was
287 used for printing artificial flowers [28], artificial zebrafish [26,40], and models of lizards [12],
288 while resin was used for printing artificial soils with fine-scale pore structure in a hydrology
289 study [31]. It is also worth considering the type of scaffolding involved with a specific printer/
290 print material combination. For some printing set-ups, the scaffolding is the same material as the
291 printed object, which means the scaffolding must be physically cut off, creating opportunities to
292 damage the printed object. Other printers are capable of dual or multi-extrusion, meaning they
293 can print using different materials simultaneously. In this case, the scaffold material differs from
294 the print material and can be dissolved after printing in a chemical solvent solution.

295 More high-tech printers capable of printing even finer-scale and more-detailed objects
296 use a powder based print material which is converted into a solid plastic with a laser. An
297 advantage of this print material is that little scaffolding is needed and extra powder can quickly
298 be removed by shaking or brushing. This media was used to print soil pore microstructure at the
299 scale of micrometers [30]. These artificial soils were printed using Nylon 12, a material that can
300 be autoclaved, which makes it possible to reuse the soils for multiple experiments [30]. Although
301 most standard printing materials are various types of plastic, there are a handful of products that
302 include other materials like wood, rubber, and metal. At least one biodegradable plastic filament
303 also exists: a polylactic acid (PLA) made from corn starch [34,41]. Perhaps the most high-tech
304 printers that have been used for an ecological application printed gelatin-based designer bacterial

305 ecosystems that varied in geometry and spatial structure in order to study cell-to-cell interactions
306 [42,43; Table 1], but this technology is not readily available to most ecologists.

307 *Printing*

308 Once the 3D image has been drafted and edited, and the printer and print materials have
309 been selected, a test round of printing is necessary before moving to the final round. Printing a
310 test object makes it possible to identify errors with the 3D image file, compare print materials
311 and confirm the material choice, and gain an estimate of the amount of time required for printing
312 *en masse*. After all aspects of the printing project have been approved, the final prints can
313 proceed. Following printing, various post-processing stages will likely need to occur, such as
314 removing scaffolding, painting, adding clay, and/or assembling pieces.

315 **Results**

316 Here we provide an example of a successful attempt to integrate 3D printing into an
317 ecological project following the workflow outlined above. We include the obstacles encountered
318 along the way as a useful case study for other ecologists. Note, we used equipment (scanners and
319 printers) and expertise from two (out of the four) 3D printing centers at our institution. For
320 ecologists with fewer onsite resources, online resources and resources at collaborating
321 institutions may be useful.

322 Clay animal models have long been used in ecological field research to infer predation
323 rates by free-ranging predators on prey. In this methodology, animal models are constructed
324 from plasticine modeling clay and then placed in the field for a fixed time period. Because the
325 clay does not harden, predation attempts leave marks in the clay, making it possible to score
326 models for evidence of predation. Early work used this method to study how body coloration
327 affected predation rates in snakes [44,45]. Since then, clay models have been used in predation

328 studies to represent a wide range of taxa including frogs [46], salamanders [47], lizards [48], and
329 insect larvae [49].

330 In many of these studies, models are constructed by hand either completely or nearly
331 completely from clay [e.g., 45,47,49,50]. In other studies, silicon molds are made from preserved
332 specimens, which are then used to make models either directly out of clay [51], or out of plaster
333 which is then covered with clay [52]. These methods clearly produce models that elicit responses
334 in predators, however, producing the models in this manner can be time consuming as studies
335 may use upwards of 100 models. In addition, modifying the models in a precise manner to test
336 the effects of prey traits on predation is difficult. The repeatability, speed, and precision of 3D
337 printing make it highly applicable to field studies of predation using models. We first explored
338 the ease of creating a 3D scan of a preserved lizard specimen, and then used software to modify
339 its body size. We then tested the durability of two print materials and two model sizes in a field
340 predation study.

341 *Making the lizard model*

342 We used two methods, a structured light scanner (David SLS-2 3D Scanner, HP Inc.,
343 Palo Alto, CA, USA) and a laser scanner (NextEngine2020, NextEngine, Inc., Santa Monica,
344 CA, USA), to make 3D scans of a preserved male *Anolis sagrei* lizard. Structured light scanners
345 operate by projecting light patterns onto the object being scanned and analyzing the pattern's
346 deformation with a camera. The laser scanner we used boasts new technology consisting of more
347 sophisticated algorithms and multiple lasers which scan in parallel, yielding more data points and
348 an overall more accurate scan. Both scanners are designed to scan 3D objects, but because they
349 use different technologies to do so, one scanner may be more effective for scanning a particular
350 object. Regardless of the number of scans or angle of rotation, the structured light scanner's

351 software was not able to converge the multiple scans into a single image of our anole, likely due
352 to the complexity and high reflectance of the preserved specimen's skin. The laser scanner,
353 however, was able to produce a digital 3D image of the specimen within about 90 minutes, and
354 we used this file going forward. The laser scanner was most successful when the lizard specimen
355 was positioned in a vertical rather than flat manner using an Extra Part Gripper (NextEngine,
356 Inc., Santa Monica, CA, USA; Figure 2A).

357 We used Maya software (Autodesk, San Rafael, CA, USA; Additional File 2) to edit the
358 scanned image (Figure 2B) of the lizard specimen to attain three goals. First, to make the lizard
359 scan possible to print, we had to edit the non-manifold geometry errors that arose due to the
360 scanning process. Second, we manipulated the size of the lizard to test whether different printing
361 materials were durable for both large and small prints. The large lizard was 25% larger than the
362 original (snout vent length = 60 mm). Finally, we added a hollow horseshoe-shaped tube in the
363 ventral side of the body cavity for looping a small wire through in order to anchor the models to
364 branches in the field. The final file we used to print the lizards is available from the authors.

365 *Print material and printing*

366 We tested two types of filament print media: plastic (ABS-P430 plastic in ivory,
367 Stratasys, Eden Prairie, MN, USA) and plastic-wood hybrid (Woodfill by ColorFabb, Belfeld,
368 the Netherlands). ABS exceeded the Woodfill in cost and perceived durability, yet Woodfill was
369 a more sustainable option as it is made of 30% recycled wood fibers. During our test print stage,
370 we learned we needed to add a base to our digital 3D image file for the Woodfill prints because
371 the scanned image was not flat which made it difficult to print. We did not need to edit it for the
372 ABS print because the scaffold base dissolved.

373 After we finalized our 3D image files from the test print stage, we printed 10 ABS
374 models on a Dimension Elite Printer (Stratasys, Eden Prairie, MN, USA) and seven plastic-wood
375 hybrid models on a BigBox 3D Printer (Chalgrove, UK) (Figure 2C). We had intended to print
376 equal numbers of each, however, the printer using the Woodfill kept getting jammed and starting
377 over, and seven was all we could print in the timeframe we had available. The printer jamming
378 was due in part to the print material and due to errors in the file geometry that were not
379 adequately resolved during the editing stage. In total, it took about eight hours to print the 10
380 ABS lizards plus an additional four hours to dissolve the scaffolding. It took nearly five days to
381 print the seven plastic-wood hybrid models (due to the printer jamming), and the scaffolding
382 needed to be cut off by hand using an Exacto knife which took about an hour for all seven
383 models. If the printer had not jammed, it would have taken two hours per model to print.

384 It was quite difficult to thread the narrow floral wire (26 gage, Panacea Products,
385 Columbus, OH, USA) through the ventral holes in both Woodfill and ABS of models. The tube
386 we made was curved, and in hindsight it should have been straight through the lizard midsection.
387 Instead, we wrapped the wire around the midsection of the bodies with two long ends hanging
388 off the ventral side. We then held the models by the wire and dipped them in melted plasticine
389 clay (Craft Smart, Irving, TX, USA) to completely cover all parts of the body and the wire
390 wrapped around the midsection. After the clay solidified (about 30 minutes), we folded the wire
391 and wrapped each lizard in aluminum foil for transport to the field.

392 In total, our time investment from scanning to printing was relatively low: it took 20
393 hours from scanning the specimen to our first test print. Additional manipulations to the image
394 took an additional 40 hours (an undergraduate working 5 hours/week for 2 months). Although
395 we had to troubleshoot issues with our image and printing, the process was relatively easy due to

396 the resources available at the 3D print centers (namely staff to mentor undergraduate on image
397 software and troubleshoot printing issues), and that we did not need the surface to be an exact
398 biological replica because we covered the models with clay.

399 *Field testing lizard models*

400 Lizard models were deployed in developed (residential yard) and natural (national park)
401 habitats on the island of Curaçao (Dutch Antilles) for 24-48h and then scored for predation. In
402 both habitat types, models were anchored to tree branches, bushes, or rocks on the ground using
403 the floral wire. We recorded evidence of predation from likely lizard and avian predators (Figure
404 2D). There were no differences in predation between ABS and Woodfill models ($F_{1,30}=0.48$, $P =$
405 0.49) or between small and large models ($F_{1,30}=0.01$, $P = 0.93$), yet more models were attacked
406 in the natural versus developed habitat type ($F_{1,30}=17.15$, $P < 0.001$) (Figure 3). We attribute
407 these differences in attack rates in habitat types to there being fewer resources available to
408 predators in natural habitats during the height of the dry season compared to more abundant
409 resources available in irrigated residential yards. Regardless, both material types were durable to
410 the field conditions and none of our models experienced any structural problems during the
411 experiment.

412 *Recommendations for using 3D printed models for field predation studies*

413 Because the Woodfill models were cheaper and just as durable as the less sustainable
414 ABS models, we would recommend using the Woodfill, or other similar plastics in comparable
415 future studies, provided that the jamming issues we encountered during printing can be attributed
416 to geometry errors in our file and not the Woodfill material itself. It should be noted that
417 although we tested the models in extremely hot ($>35^{\circ}\text{C}$) field conditions, we cannot comment on
418 the durability of the two materials in rainy or very cold conditions. Initially, we believed the

419 Woodfill would crumble more on the smaller model with narrower appendages, but this was not
420 the case. Finally, our study took place over a 3-week period. It is possible that over longer time
421 periods, the Woodfill would not be as durable as the ABS plastic.

422 **Discussion**

423 *Reduce, Reuse, Recycle*

424 While 3D printing can facilitate ecological research, the use of this technology must be
425 weighed against its environmental and social costs. In general, 3D printing has the potential to
426 reduce CO₂ emissions and lead to more sustainable practices in the consumer manufacturing
427 industry [53], yet there are still many less sustainable aspects to consider. Three-dimensional
428 printing is much more energy intensive than 2D printing, and the most widely used materials in
429 3D printing, plastics, are fossil fuel based. Most 3D materials (e.g., plastic filament) are virgin
430 and not derived from recycled post-consumer products, and if not disposed of and recycled
431 properly, printed objects can exist in the environment for ages. The plastics used in printing can
432 be toxic to aquatic organisms, especially resin-based printed objects [54]. The printing process
433 itself generates extra waste due to printers jamming, misprinted models, and scaffolding
434 necessary for more complex 3D objects. In addition, printing produces harmful emissions in the
435 form of ultra-fine particles and volatile organic compounds [55,56], which can be especially
436 worrisome as many 3D printers are housed in indoor office settings [57]. With respect to the
437 manufacturing of any plastic item, these negative aspects are not unique to 3D printing, they just
438 become more obvious when one is directly involved in the manufacturing process. In our
439 specific case, we chose 3D printed models for the speed at which they could be produced and
440 their durability as we intend to use them in future experiments. Ecologists planning to

441 incorporate 3D printing in research should strongly consider the negative impacts associated with
442 3D printing compared to the impacts of creating objects via other methods or not at all.

443 There are promising advances in the sustainability of 3D printing materials. Materials
444 scientists are developing a range of filaments that are biodegradable, compostable, and made
445 from recycled materials. For example, Eco-Filaments, such as WillowFlex (BioInspiration,
446 Eberswalde, Germany), are made from plant-based resources and are completely compostable,
447 even in residential compost bins. Other filament choices are made from recycled plastics like car
448 dashboards, PET bottles, and potato chip bags (3D Brooklyn, Brooklyn, NY, USA; Refil,
449 Rotterdam, the Netherlands). In fact, the cost of generating recycled plastic filament is often less
450 than filament made from raw materials, prompting the establishment of a fair trade market for
451 used plastic collected by waste pickers in the developing world (e.g., Protoprint Solutions, Prune,
452 India) [58]. Non-plastic recycled filament options exists, such as filament made from the waste
453 products of beer, coffee, and hemp production processes (3DFUEL, Fargo, ND, USA) as well as
454 wood pulp [59]. Finally, because common print materials such as ABS plastic are not
455 biodegradable or recyclable in municipal recycling centers, machines have been developed to
456 recycle these plastics directly at the printing site [60]. These machines grind old prints and melt
457 them into new filament that can be reused for printing (e.g. Filastruder, Snellville, GA, USA).
458 Across sustainable options for print materials, we can attest to the durability of Woodfill for
459 applications comparable to ours. For ecologists considering other sustainable print materials,
460 most of these companies readily provide information about the durability of their products.

461 We stress that all 3D printing projects in ecological research should reduce, reuse, and
462 recycle: *Reduce* the amount printed and the use of toxic print materials; *Reuse* printed objects
463 and use materials made from post-consumer, waste materials; and *Recycle* printed objects by

464 choosing materials that can be easily recycled, composted, or that are biodegradable. Planning a
465 print job (Fig. 1) requires both careful estimation of the minimum number of replicates to print
466 and smart design of geometry that minimizes or eliminates scaffolding, as scaffolding is usually
467 discarded. Printing should be performed in well-ventilated environments where airborne toxins
468 do not accumulate and harm personnel. The environmental toxicity of objects should be reduced
469 by choosing materials with low toxic potential and reducing the toxicity of materials post-print.
470 For example, exposure of resin-based printed objects to intense UV light can reduce their
471 toxicity to aquatic organisms [54]. Printed objects should be reused in research as much as
472 possible to avoid repeat printing, and print materials made from recycled material or materials
473 that are recyclable or compostable should be used when possible. While most ecologists will not
474 invest in their own 3D printing equipment and instead employ general-use academic (e.g.,
475 library) or commercial facilities, these environmental concerns can be communicated to the
476 printing facilities so that they might adopt sustainable practices in their 3D printing for research.

477 **Conclusions**

478 In conclusion, 3D printing technology has the promise to reduce the time and cost
479 invested in creating custom materials used in ecological research, while at the same time
480 increasing the ease at which collaborations occur within and outside the scientific community.
481 Although there is a learning curve for developing 3D image files, there are ample online libraries
482 of 3D files, plus tech savvy students and 3D printing center staff can be extremely helpful.
483 Recent advances in print materials may reduce the footprint associated with this new technology.
484 Overall, as with any new technology, ecologists must weigh the costs in terms of time and
485 monetary investments into developing usable 3D materials for research versus other methods of
486 generating those materials. If ecologists are in the position to commit the initial investment in

487 securing printing resources and navigating the technological learning curve, the resulting ability
488 to implement 3D printing into future studies could save time and money on the long term.

489 **Declarations**

490 **Ethics approval**

491 All work conducted involving live animals was in accordance with the Institutional Animal Care
492 and Use Committee at Temple University (IACUC protocol #4614).

493 **Consent for Publication**

494 Not applicable

495 **Availability of data and materials**

496 The datasets used and/or analysed during the current study are available from the corresponding
497 author on reasonable request.

498 **Competing interests**

499 The authors declare that they have no competing interests

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503 **Author Contributions**

504 JB and MH conceived of and designed the study; JB, BW, and MH developed the workflow; BW
505 tested the workflow and designed the 3D model; JB and MH conducted the field experiment; JB,
506 BW, STH, and MH wrote the manuscript and provided editorial advice. All authors read and
507 approved the final manuscript.

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513 **Additional Files**

514 **Additional File 1.** Software for designing, modifying, and analyzing 3D files

515 **Additional File 2.** Online libraries of 3D imagery relevant for ecological research (as of 2017)

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517

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- 662

Table 1: Ecological studies that have used 3D printing

Research Topic	Taxa	Objects Printed	Print Medium	Sample Size ^a	Reference
<i>Behavioral Ecology</i>					
Egg rejection behavior in context of brood parasitism	brown-headed cowbird (<i>Molothrus ater</i>)	cowbird eggs that varied in size/shape, then painted different colors	“white strong and flexible plastic, polished”	80	[25]
Effect of corolla shape on pollinator behavior	Hawkmoth (<i>Manduca sexta</i>)	flowers that varied in corolla shape based on specific mathematical parameters	Acrylonitrile butadiene styrene (ABS) plastic	NR	[28]
Effects of visual and olfactory floral traits in attracting pollinators	Mushroom-mimicking orchid (<i>Dracula lafleurii</i>)	molds to make silicon flowers	Cyanoacrylate impregnated gypsum	NR	[29]
Effect of nectar caffeine concentrations on pollination service	Bumble Bees (<i>Bombus impatiens</i>)	structures that functioned like corollas over glass jars containing artificial nectar	Plastic (type non-specified)	Min. 18	[27]
Social behavior of zebrafish in response to varying stimuli	Zebrafish (<i>Danio rerio</i>)	predatory fish model robot	ABS plastic	1	[61]
		shoals comprising 3 zebrafish that varied in body size plus anchoring materials	ABS plastic	4 shoals	[26]
		biologically-inspired zebrafish replica	ABS plastic	1	[40]
Evaluation of 3D printing as suitable method for field predation model studies	Brown anole (<i>Anolis sagrei</i>)	lizard models using 2 print media, covered in clay, and field-tested for predation	ABS plastic, plastic-wood hybrid filament	17	This study

Thermal Ecology

Comparing thermodynamics of 3D printed and copper lizard models	Texas horned lizard (<i>Phrynosoma cornutum</i>)	thermal models of lizards	ABS plastic	10	[12]
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Tools – Experimental arenas

Evaluation of 3D printed soil as suitable for fungal colonization	Plant pathogenic fungus (<i>Rhizoctonia solani</i>)	artificial soil from 3D scans of soil with varying micropore structure	Nylon 12	10	[30]
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Comparing hydraulic properties of 3D printed soil relative to real soil	Soil	artificial soil from 3D scans of soil	Resin (Visijet Crystal EX 200 Plastic Material)	14	[31]
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Microscale bacterial cell-cell interactions	<i>Pseudomonas aeruginosa</i> and <i>Staphylococcus aureus</i>	“designer” bacterial ecosystems that vary in size, geometry and spatial distance with exact starting quantities of <i>P. aeruginosa</i> and <i>S. aureus</i>	Gelatin	NR	[42,43]
---	--	--	---------	----	---------

Effect of interstitial space on predator-prey interactions	Blue crab (<i>Callinectes sapidus</i>) and Mud crab (<i>Eurypanopeus depressus</i>)	oyster shells aggregated into artificial reefs that varied in interstitial space configuration	Polylactic or ABS plastic	NR	[33]
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Tools – Sampling Equipment

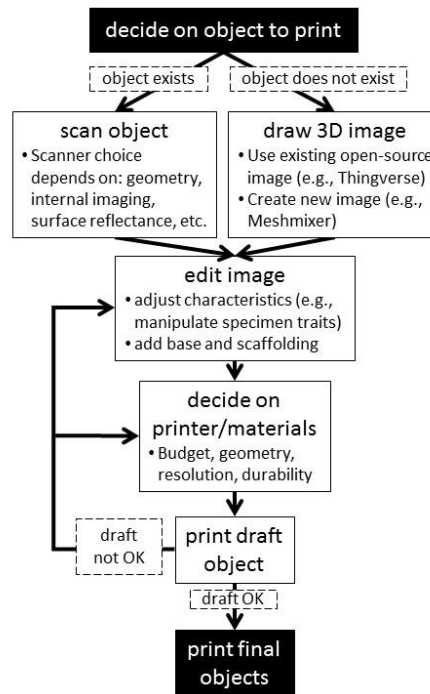
Collecting unobtrusive biological samples from whales	Southern right, humpback and sperm whales	components to build an unmanned surface vehicle for oceanographic research (<i>SnotBot</i>)	ABS plastic and nylon	1	[36]
---	---	---	-----------------------	---	------

Tools for studying the impact of ambrosia beetles on trees	Shot hole borer beetle (<i>Euwallacea fornicatus</i>)	components for entry devices and emergence traps	ABS plastic	15	[35]
Testing decoys vs real beetles to enhance trap capture rates	Emerald ash borer beetle (<i>Agrilus planipennis</i>)	beetle decoy to use on traps	ABS plastic	300	[11]

^aNR = Not reported

664 **Figure Legends**

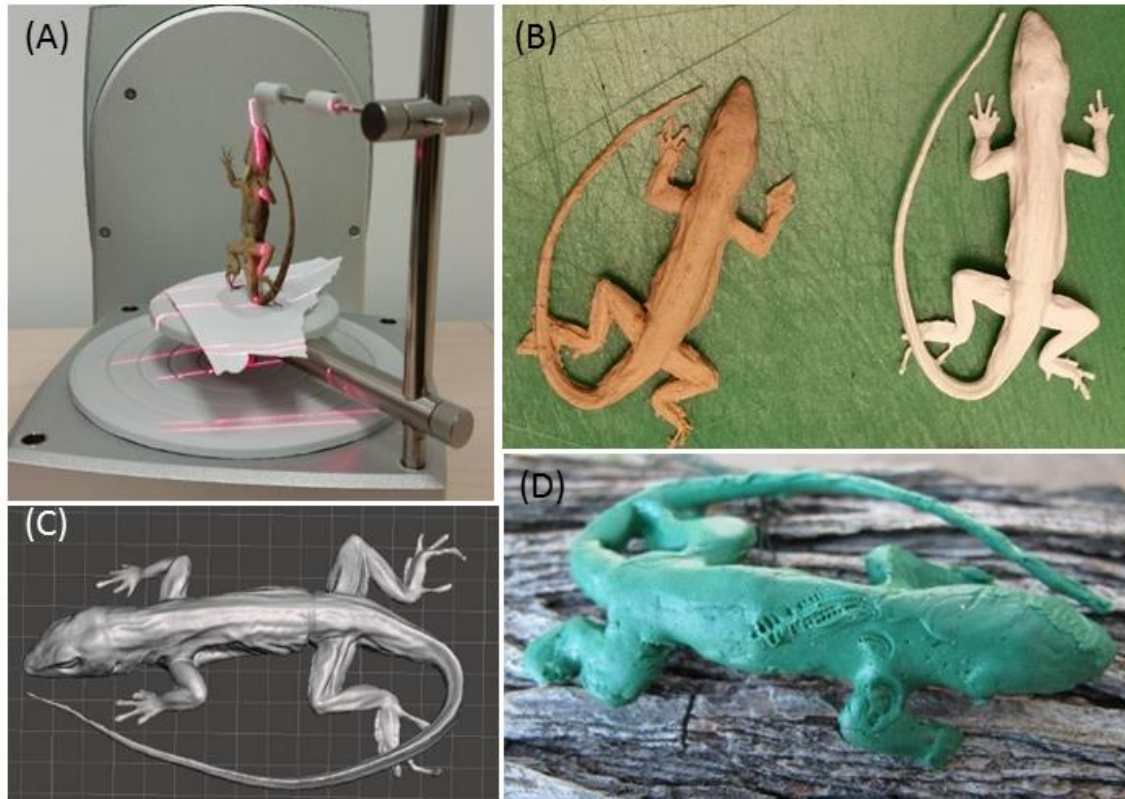
666 **Fig. 1** Workflow illustrating steps for integrating 3D printing in ecological research



668

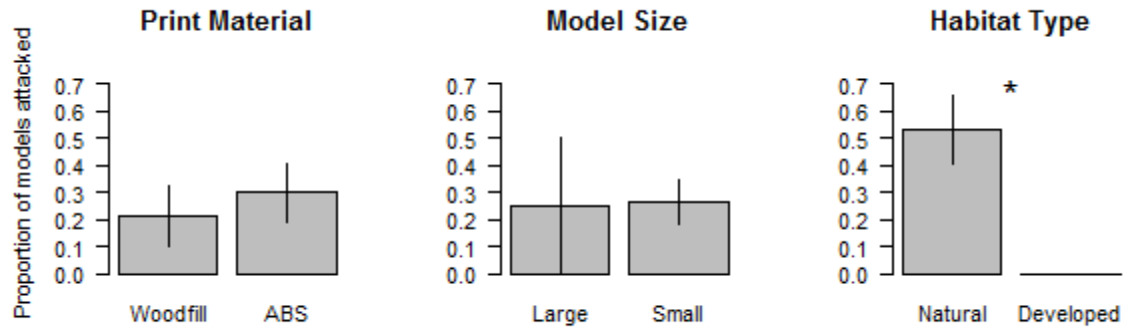
670

Fig. 2 Construction of a 3D printed lizard predation model a) Successful laser scanning setup of
672 preserved brown anole (*Anolis sagrei*) specimen in vertical orientation; b) 3D image of scanned
anole viewed in Meshmixer software and later edited in Maya; c) 3D printed plastic-wood hybrid
674 (left) and ABS plastic (right) anole models; d) clay covered model on a branch in the field with
bite marks likely from a lizard predator (*Cnemidophorus murinus murinus*).



676

678 **Fig. 3** Proportion of clay-covered 3D printed models attacked during field predation trials. Bars are means
+/- one standard error; * indicates significantly different means at $P < 0.01$.



680